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BOTANICAL SCIENCES

EDMUND W. SINNOTT, *Consulting Editor*

Principles of
GENETICS

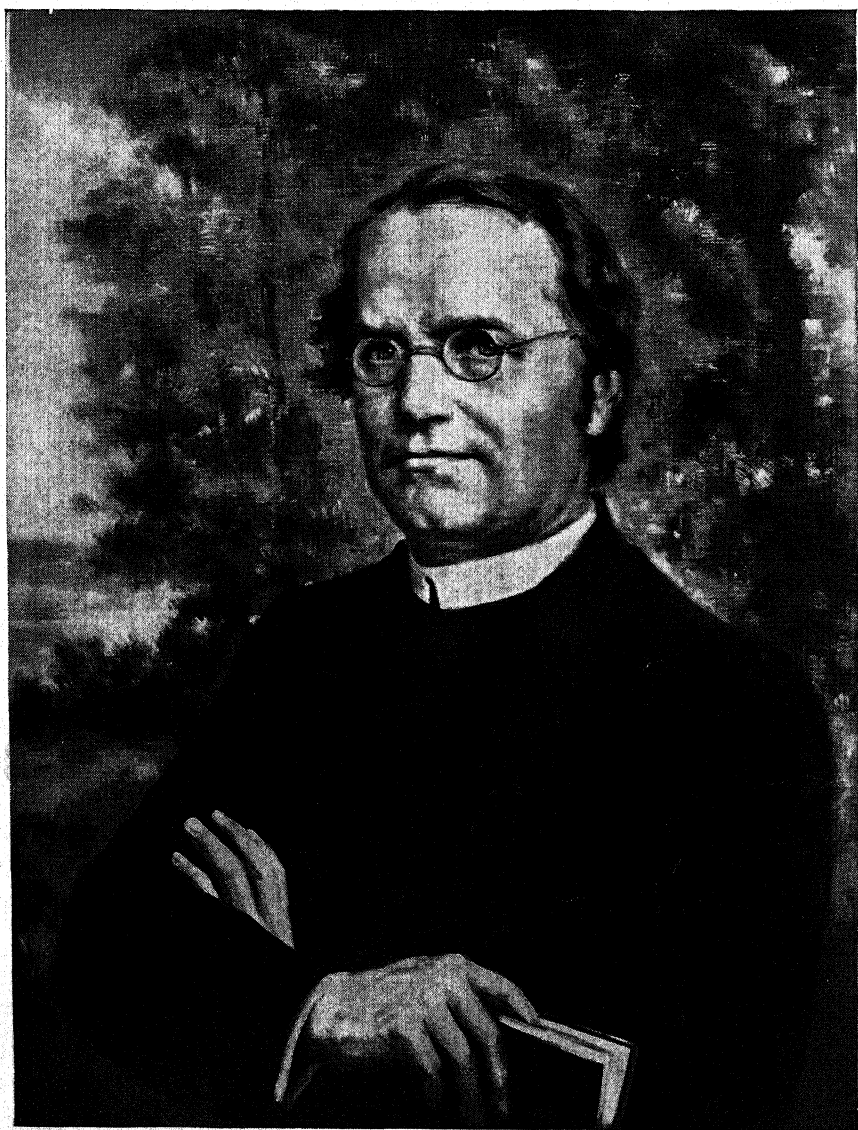
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The founder of genetics, Gregor Johann Mendel, 1822-1884, from the painting by Flannery.
(Courtesy of Dr. Hugo Illis.)

Principles of G E N E T I C S

By EDMUND W. SINNOTT
*Sterling Professor of Botany and Director of the Sheffield
Scientific School, Yale University*

L. C. DUNN
Professor of Zoology, Columbia University

TH. DOBZHANSKY
Professor of Zoology, Columbia University

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FOREWORD

There is a common feeling that a textbook is a full and final exposition of the subject which it treats, and that by virtue of "knowing the book" one acquires all the knowledge of the subject which it is necessary to have. Such beliefs have little to justify them. No text is or can be complete or final; nor, if it were, would an understanding of the subject be gained by committing the whole book to memory. Knowledge is not acquired in this way, but grows in the minds of those who discover for themselves new facts and relationships.

The principles of genetics have developed out of the arduous study of scores of investigators, and understanding of principles can best be gained by the student through a process which is somewhat similar to that employed in their original discovery. This process begins with, and is continually stimulated by, curiosity as to the methods and the mechanism of inheritance; it proceeds by the collection and study of facts, and by a critical discrimination between those which are true and relevant and those which are untrue or irrelevant; and finally it involves a considerable practice of the reasoning faculty by which deductions are made, and applied or tested on many similar cases. It is only in this way that the process of inheritance can be *understood*. The learning of facts alone cannot accomplish this.

As an aid to such a comprehension of the science of genetics, this book includes problems of two types, which form an integral part of the subject matter. These are designed to stimulate curiosity, to provide opportunity for practicing and extending the methods and applying the theories outlined in the text, and to point the way to other related facts not specifically treated in this book. They are not designed as memory tests, although the continual use of facts in solving problems is at once the best method of committing these facts to memory as well as of understanding them.

One of these aids consists of questions for thought and discussion. Answers to these are not to be found in the text itself, but may be reached by a process of reasoning for which only the premises are given. Familiarity with the subject matter of the text will provide the raw material, while the synthesis resulting in a correct answer or intelligent discussion must take place in the student's mind.

Other problems are designed to provide more extended practice in reasoning from principles. Nearly all of this type require some computation

and may be most profitably studied as laboratory exercises under the guidance of an instructor. It is desirable to use labor-saving or "short-cut" methods (such as the checkerboard method described on page 63) wherever possible, in order that the mechanical work involved in calculation may not be regarded as the chief benefit to be derived from the problems. Sufficient information for solving all of them is contained in the text or in the supplementary notes in the problems.

The references cited will aid the student in examining the original publications from which our knowledge is derived. Of these Mendel's paper is still the most important and can be read with interest by all students. Citations of current literature are not intended to be complete; they should, however, indicate to the student that the subject as a whole is not contained in the text but is growing by the continual accretion of reports of experiments, all of which do not yield results in entire consonance with the few points of view which it is possible to present in a brief textbook. Some of the references will lead to new material not mentioned in the text, which must be reconciled with the fundamental principles of genetics, while others may serve to make connections between the student's knowledge of genetics and his experience in other directions.

PREFACE

The fourth edition of this textbook was called for not only by the many additions to our knowledge of genetics which the past ten years have brought but also by the need for correcting and strengthening those sections of the third edition which both we and many of our colleagues had recognized as inadequate.

Although the entire book has been rewritten, we have tried to preserve its essential character as a general introduction to principles and the actual problems of genetics. The chief changes will be found in the chapters dealing with the physical basis of heredity in the chromosomes (Chapters VII through X). We have described the chromosome mechanism not for its own sake but as a genetical system, the transmission apparatus of heredity, and have described the departures from the normal structure and functioning of this system (chromosome aberrations) in some detail because from such abnormalities proofs of the actual arrangements of genes in chromosomes, the cytological maps, can be obtained.

Most of the new material added concerns the fields of special interest to the two authors chiefly responsible for the revision, namely, population genetics and speciation on the one hand, and on the other, the mechanisms of genic effects on development. It happens that the chief recent expansions in our knowledge of genetics have occurred in these fields. We have devoted a good deal of space to the use of fungi such as *Neurospora*, and microorganisms such as bacteria, protozoa, and viruses, which has opened up possibilities for understanding the mode of action of genes. The analysis of the breeding structure of natural populations in both animals and plants and of human populations has required the insertion of three chapters (XII, XIII, and XIV) dealing with the general principles of population genetics based on the Hardy-Weinberg equilibrium, on race formation, and on speciation.

Throughout the book the use of material and examples from human heredity has increased considerably over that in previous editions. Instead of separating this from the other materials of genetics we have treated it as a part of a consistent whole, for heredity in man differs in no essential way from heredity in other animals and plants.

The problems have been revised and many new ones introduced, especially in the chapters on chromosomal aberrations and population genetics.

As an appendix we have included a translation of the original report of Mendel (1865), with which modern genetics began. We believe all students of genetics should be familiar with this paper which still forms the best introduction to the basic evidence and the principles derived from it. The translation was made by the Royal Horticultural Society of London, and we are grateful to the council of the society for permission to reproduce it.

We are indebted to many colleagues for their courtesy in supplying new illustrations as acknowledged in the captions, and particularly to Professor M. M. Rhoades for criticism and for new material prepared for this edition, including the photomicrographs of meiosis in maize and the linkage maps of maize. We acknowledge with special gratitude the aid and patience, in connection with the preparation of the manuscript, of Natalie Sivertsev Dobzhansky, Louise Porter Dunn, and Pauline Goldman.

THE AUTHORS

April, 1950

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CHAPTER I

GENETICS, THE SCIENCE OF HEREDITY AND VARIATION

Between those things which are alive and those which are lifeless there exists a gap which science has not yet bridged. Living beings which are so various as to include man and the higher and lower animals, the great plant kingdom, the microorganisms, and the smallest forms of all, the viruses, all share certain common properties which distinguish them from lifeless matter. One of the most important of these properties is self-reproduction. The organized unit in which living matter always occurs—that is, the living individual—must always arise from some preexisting living individual and never from lifeless matter itself.

The classical work of Spallanzani and of Pasteur gave the deathblow to the old belief in the "spontaneous generation" of living things out of dead material and proved that even among the most minute organisms the spark of life can be kindled only by life itself. Recent studies on viruses which multiply in the living cells of plants and animals, seem to have narrowed somewhat the gap between the living and the lifeless, since, under the electron microscope, aggregates of some viruses appear as crystallike bodies. Yet, so far as we know now, individual virus particles arise only from living virus. Every organism now living is therefore to be looked upon as the latest member of a long and uninterrupted succession of living beings, extending back, generation after generation, to the dawn of life. This is the essential teaching of the theory of evolution. The actual origin of life itself is lost in the mists of antiquity, but the pageant of the evolutionary history of living things, which unfolds itself in the fossil record of ancient times, makes it clear beyond any reasonable doubt that the animals and plants of today are direct lineal descendants of earlier and more primitive types. Continuity is of the essence of life.

Reproduction. Since individual living things grow old and die, however, this continuity must be maintained by the transmission of life from one individual to a succession of new ones, its offspring. This process is known as *reproduction* and may take place in various ways.

In the simplest methods, commonly called *asexual*, or *vegetative*, reproduction, the body of the parent becomes divided into two or more parts, each of which grows into a new individual. With animals this method is uncommon except in the simplest types, but among plants the fact that a

small portion of the body, when removed and placed under favorable conditions, will often restore the missing parts and establish itself as a new individual makes multiplication of this type easy and effective both in nature and through the various arts of plant propagation.

Far commoner and more important than this asexual, or vegetative, method is that called *sexual reproduction*. An essential feature here is that the new individual arises through the union of two sex cells, or *gametes* which form one cell, or *zygote*, from which develops the new individual. The successful consummation of this process is ensured by a great variety of structures and functions found throughout the animal and plant kingdoms. In the lowest plants and animals the gametes which unite may be so much alike that neither of them can be classed as female or male. In most organisms, there is a division of labor between relatively large, food-laden female cells, the *eggs*, or *ova*, and small and usually motile male cells, known as *sperms*, or *spermatozoa*. In the higher plants a series of complicated reproductive structures—the flower, fruit, and seed—have been evolved. The male gametes are here produced within *pollen grains* and the female gametes within the *ovules*. The fertilized egg develops into the embryo of the seed.

Heredity. The single gamete which a parent contributes to each of its offspring is usually too small to be visible to the unaided eye (Fig. 1). Yet this extremely narrow bridge is the only direct physical link between parents and offspring, and across it everything must pass which is *transmitted* from one generation to the next. Muller has estimated that all the spermatozoa from which the present population of the world arose (more than 2 billion individuals) would make no larger bulk than half of an ordinary aspirin tablet. The essential parts, that is, the chromosomes, of 2 billion eggs would occupy about the same space. This minute amount of substance has nevertheless determined, in cooperation with the factors of the environment, the kinds of human beings which inhabit the earth. Indeed, these tiny containers of reproductive substance, into which so much is packed and out of which so much emerges, are the most remarkable bits of matter in existence.

Regardless of whether an organism reproduces sexually or asexually, the bit of the parental body which gives rise to the new individual undergoes growth. The body of an adult man has a mass tens of billions of times greater than that of the fertilized egg from which it has developed. In order for growth to occur, much material has evidently to be taken from the environment and incorporated into the developing body. In the case of green plants, this material consists of water and mineral salts taken from the soil and of carbon dioxide and oxygen from the air, the energy of sunlight making the assimilation of these other materials possible. In living

things other than green plants, growth takes place by the incorporation into the body of whatever organic and inorganic foods the organism requires.

The food taken in from the environment becomes a part of a living body

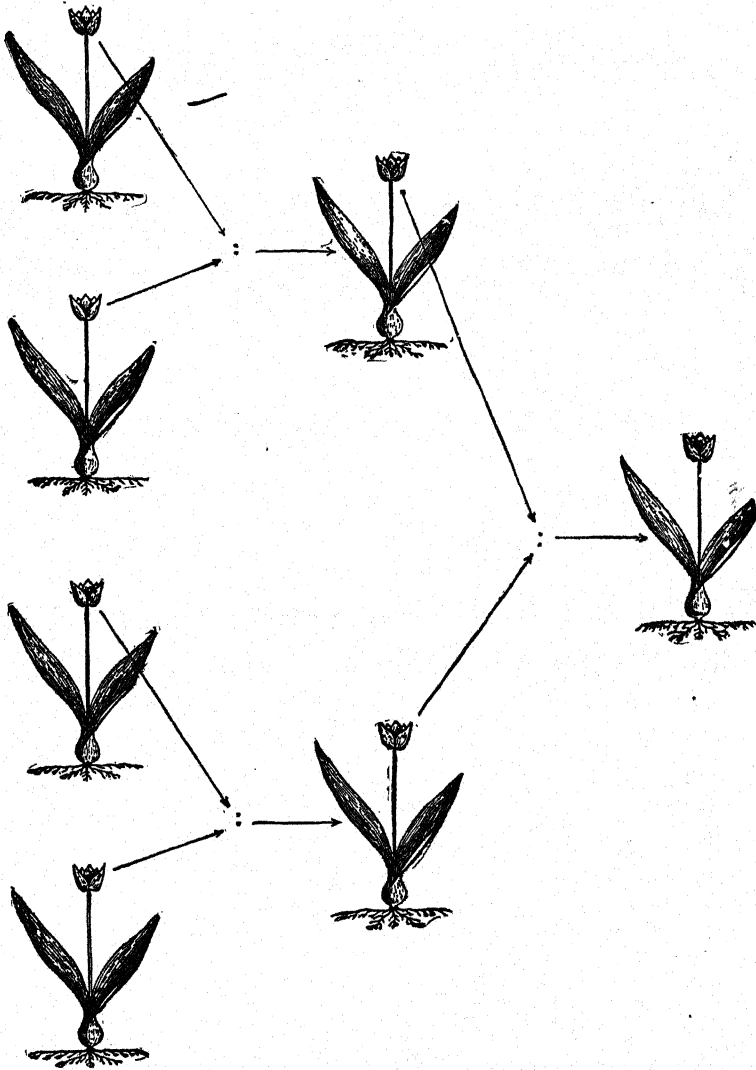


FIG. 1. The narrow hereditary bridge. The plant at the right receives from each of its parents only one minute sexual cell, a male gamete from one and a female gamete from the other. The parents, in turn, receive from each of the grandparents but one sexual cell. Thus the bridge which connects one generation with the next, and over which the entire inheritance must pass, is an exceedingly narrow one.

only through profound chemical and physical transformations wrought by the body upon the substances in the food. Moreover, and this is fundamental, every organism grows by transforming its food in a definite way, so that the outcome of the growth process is always a more or less faithful copy of the bodies of the parents and other ancestors of the developing individual. Thus, the parental organism reproduces itself in its offspring by causing it to organize, in the same definite way, the materials taken in from the environment; and this process of self-reproduction is the essence of *heredity*. It is because of heredity that individuals related by descent resemble each other.

Variation. Heredity does not necessarily mean that parents and offspring are completely identical. Probably no two human beings or two individuals of any other species are ever exactly alike. This is, first of all, because the environment of organisms is never the same in different places and at different times. No two plants which grow side by side in a meadow receive precisely the same amounts of light, water, and minerals; no two animals receive quite the same food at the same stage of development. Two individuals with the same heredity may become somewhat different when they come into contact with different conditions of food, temperature, light, humidity, and other external factors. Such differences among organisms of similar heredity are referred to as *environmental variations*, or *modifications*.

The second cause of unlikenesses is that there are many different kinds of hereditary constitution. Although each kind tends to reproduce itself faithfully from generation to generation, changes in heredity occasionally do occur. These changes, called *mutations*, are perpetuated in the offspring, because the altered heredity reproduces itself just as faithfully as the original condition did. It will be shown later that the hereditary substance is composed of discrete parts, called *genes*, and that different genes may undergo mutation independently. In the process of sexual reproduction, different genes become associated with each other in various combinations. *Recombination* is one of the commonest sources of hereditary variability. The numbers of these combinations may be so enormous that, in most sexually reproducing species including man, probably no two individuals (except identical twins, see page 20) have exactly the same heredities. The diversity which is produced by differences in heredity is described as hereditary variation, or *genotypic variation*. Environmental and hereditary variations occur in nature side by side, so that the differences observed between individuals are usually partly environmental and partly hereditary in origin.

Evolution. Because of its peculiar hereditary constitution, each organism from the lowest virus to the highest animal tends to transform the

materials from the environment into its own likeness; and as reproduction continues and the descendants increase, the process exercises a "pressure" on the environment. This pressure tends to transform the materials available in the world into bodies of a particular kind of organism. The first living thing that appeared on earth started this trend toward converting nonliving into living matter.

The rates of reproduction differ widely in different organisms. Some bacteria divide once in about twenty minutes. The length of a generation in man is about a quarter of a century, and the number of offspring produced by a pair is small. A sequoia tree lives for three thousand years or more and produces numerous seeds. Nevertheless, any organism tends to multiply until it exhausts the available food supply, occupies all the accessible space, or is checked by enemies or parasites. Each species can use only certain substances for food and can inhabit only certain climates and soils. To be sure, some forms of life are more and others less narrowly specialized in food and habitat requirements. Thus, some parasitic protozoans live only in the blood stream of a single species of a mammal or a bird; on the other hand, the creosote bush, *Larrea divaricata*, grows apparently equally well below sea level or very high in the mountains. However that may be, the populations of any species of organism tend to expand until the expansion is checked by the inertia of the inorganic environment.

We have seen that the conservatism of heredity, which makes like beget like, is opposed by mutation, which produces new hereditary variants. Some of these variants may be able to use as food, substances which are not used or which are exploited less efficiently by other organisms. One type of variant may reproduce faster than others under certain climatic or soil conditions. If the new variant reproduces more efficiently, it will gradually supplant the ancestral form; or else both the original and the ancestral forms will exist, each in the sphere in which it is most competent. In either case, there will have appeared in the world a new form of life. On the other hand, a variant which reproduces less efficiently than the original form (and most variants that arise by mutation are less efficient) will be eliminated. The propagation and spread of the efficient and the nonperpetuation of the inefficient hereditary variants constitute what is known as *natural selection*.

Heredity modified by one mutation may be changed again and again by other mutations. Darwin in "The Origin of Species," published in 1859, developed a theory which, in our modern terminology, may be stated as follows: Natural selection, acting on a succession of hereditary variants produced by mutation, leads to a gradual change, or *evolution*, of organisms.

It may also be said that evolution is a necessary outcome of the interaction of two opposite forces: heredity, the conservative agent which makes

similar organisms reappear generation after generation, and variation (mutation), through which novel types of heredity arise from time to time. Natural selection, which acts as a guiding factor in evolution, is, in the last analysis, a consequence of self-reproduction in a limited environment. The first self-reproducing substance which appeared in the world must, consequently, have carried in itself the potentiality of evolutionary change. Self-reproduction seems to be the fundamental property of life.

Development. The processes underlying self-reproduction constitute one of the central problems of biology which is far from having been solved at present. A fertilized animal egg or a fertilized plant egg contains all the hereditary factors which will interact with the environment to produce a body of its own species and variety. To that extent, the body is pre-determined or preformed in the fertilized egg. On the other hand, we find a hen's egg contains nothing which we can recognize as resembling an adult hen, and one of maize has nothing reminiscent of a maize plant with its roots, stem, leaves, and inflorescence. A hen or a maize plant arises from the egg by means of a succession of certain processes and structures which follow one another in a definite order. These transformations constitute a true development to which the term *epigenesis* has been applied. It is essentially the gradual creation of something which did not exist before except as a potentiality.

In the eighteenth and nineteenth centuries, when biology was still in its early infancy, some authorities looked on development as pure *preformation* or pure *epigenesis*. Some imagined that they saw a diminutive human figure inside of the head of human spermatozoon, and they even pictured this imaginary figure. On this view, development would be nothing more than the expansion of this preformed human frame. Nowadays, however, it is generally admitted that development partakes of both preformation and epigenesis.

Genetics. Bateson in 1906 proposed the name *genetics* for that branch of biology concerned with "the elucidation of the phenomena of heredity and variation." Genetics is one of the younger members of the family of biological sciences, for as a distinct science its history goes back only to the year 1900. Progress in the development of genetics has, however, been extremely rapid, and it is still proceeding apace.

Of course, from the earliest times men recognized the fact that "like begets like." They used this fact, more or less unconsciously, perhaps, in choosing for breeding purposes those individuals among their domesticated animals and plants which best suited their requirements and speculated about the possible causes of heredity and variation. Thus, the writings of Aristotle (384-322 B.C.) contain descriptions and interpretations of genetic phenomena which are at present of interest only as historical curiosities.

A new impetus to studies on heredity was given by the invention of the microscope and by the clarification of the mechanism of sexual reproduction. Male cells, spermatozoa, were recognized in the latter half of the seventeenth century, and their function as initiators of development in the egg was demonstrated experimentally, by Spallanzani in Italy, early in the eighteenth century. In the plant kingdom the very fact of sexuality was long unknown, until Camerarius concluded in 1694 from experiments with plants that their reproduction also followed the sexual method known in animals, with the pollen functioning as the male, the ovule as the female, element. In 1760 the German botanist Kölreuter (Fig. 2) performed the first careful experiments in plant hybridization, crossing two species of tobacco by placing the pollen of one on the stigmas of the other. The offspring resulting from this experiment were intermediate in most respects between the two parent species, thus proving not only that pollen performed an essential function in seed production but that parental characters were transmitted both through the pollen and through the ovules.

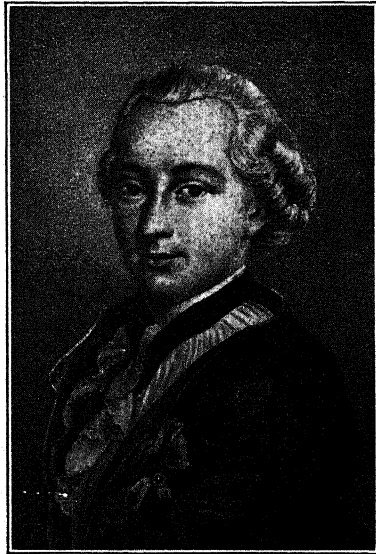


FIG. 2. Joseph Gottlieb Kölreuter (1733-1806). (Courtesy of Genetics.)

Everyday experience shows that children are, as a rule, intermediate between their parents in many of the traits in which the parents differ from each other. Thus, first-generation mulattoes appear to be in most respects about halfway between their Negro and their white ancestors. The fact that parental characteristics often seem to be blended in the offspring once led, in the popular imagination, to the conjecture that the parental heredities, or "bloods" as they are frequently but misleadingly called, were also blended in the child. It was as though the parental heredities behaved like miscible liquids, such as alcohol and water, which could be dissolved in each other. According to this view, the heredities of the parents were, in turn solutions or alloys of the grandparental ones, the heredities of the grandparents were mixtures of equal parts of the great-grandparental ones, etc. In short, the heredity of any individual would be a blend in equal parts of the heredities of all ancestors of any given generation.

Blood plays, of course, no role in the transmission of heredity. Never-

theless, the blending concept of heredity is still current among laymen, and in the nineteenth century it was accepted by biologists as well. Francis Galton, a cousin and a younger contemporary of Darwin, even gave a mathematical expression of heredity based on this view, which he called the "law of ancestral heredity." The theory was invalidated in 1866 by the publication of results of experiments which proved conclusively that the heredity transmitted from parents to offspring consists of discrete unit particles. This was the essence of the great discovery of Mendel. Later these particles became known as genes. Genes are unlike "bloods" or any miscible liquids; they preserve their individualities and their differences despite being packed closely together in the cell nuclei.

In sexual reproduction, the individual, or *zygote*, is a double structure, which is formed by the fusion of two *gametes*, one (the egg) from the mother, the other (the sperm) from the father. Each gamete contributes *one* of each kind of gene from the mother and *one* of each kind from the father. A zygote carries, therefore, every gene in duplicate. These parental and maternal genes do not, however, fuse or amalgamate. On the contrary, when the individual forms its own gametes, the paternal and the maternal members of each pair of genes segregate and pass to different gametes. Each gamete carries, therefore, only one half of the genes which an individual possesses. Different paternal and maternal genes segregate independently, and therefore different gametes produced by the same individual contain somewhat different sets of genes. Brothers and sisters are thus likely to receive different sets of genes from their parents.

Important as Mendel's work has proved to be, it was not recognized as such by scientists of his day. It was only in 1900 that three biologists independently recognized the importance of Mendel's paper and made it known to the scientific world. The year 1900 is, therefore, taken as the beginning of the existence of genetics as a separate branch of biology.

What Is Inherited. Resemblance of parents and offspring is a very general phenomenon. In the first place, there is no exception to the fact of similarity between parent and offspring in *general* features. We have no difficulty in recognizing the "humanness" of every child that is born or in identifying the descendants of a maize plant as members of that species. The success of naturalists in classifying the animals and plants of the world in an orderly system in which each individual has its assigned place, and the practical rule which has guided them, namely, that individuals which look most alike are probably most closely related, both bear witness to the general truth of hereditary resemblance. Moreover, resemblance extends to many of the more special structural and functional features of organisms which are shared by members of the same species, variety, or local race. Thus we can identify the progeny of purebred fox terriers not only as mam-

mals, carnivores, and canines but as members of a single variety of dog marked by features of size, color, behavior, and other traits which they share with their parents and relatives. This resemblance may extend to those relatively minor characters by which we infer the relationship between brother and sister or father and son—the color of eyes or form of hair and similar evidences of the inheritance of single features. The traits affected by heredity may be of many diverse kinds, appearing at all stages of the life cycle. Gross structural features such as size, shape, and color are, of course, the most obvious, but it is perhaps more significant that the heredity received from the parents determines, in plants, the kinds of proteins and carbohydrates and even the special characteristics of the starch grains which are formed, while in animals it fixes the chemical peculiarities of the blood and the tissues. Heredity likewise influences the physiological reactions of the organism. It decides whether an individual or a race of plants will be parasitized by a fungus of one variety or of another; whether an insect is to survive successfully at a higher or at a lower temperature; whether or not a child is to distinguish all of the colors of the spectrum or only certain of them, that is, whether he is to be color-blind; whether a plant is to convert some of the soluble sugars which it manufactures into red or blue or brown pigments or into no pigment at all. These diverse peculiarities influenced by heredity in turn influence others in the organism and affect its relation to its surroundings and its chances of survival in nature. Certain caterpillars of one species of butterfly absorb into their body fluids the several components of chlorophyll, the green coloring matter of leaves, so that they appear green like the leaves on which they feed. Others of the same species with a slightly different heredity can absorb only specific parts of chlorophyll and assume a blue-green color. Those of the former sort are less likely to be discovered by their natural enemies, the birds, than are the latter, since they are less conspicuous on green foliage.

In short, all kinds of traits are influenced by heredity. The fact that parents and offspring resemble each other in a certain trait, say in shape of the head or in hair color, is often stated by saying that head shape or hair color is transmitted by heredity. Such a statement is convenient because it is short and concise, but it leads to confusion and misunderstanding if taken too literally. Indeed, gametes contain nothing resembling the head of an adult man and nothing resembling hair of any color. It is evident that heads and hair are not “inherited” as such. Yet the gametes do contain something which enables the developing organism not only to form a head covered with hair but which induces this head to assume a certain shape and the hair to acquire a certain color.

A little consideration will show that what is inherited is, in each case, the ability to react in a specific way to specific environmental factors. This is

obvious in the case of hereditary susceptibility to disease. If a plant inherits a constitution which fits it to be the ideal home for one variety of fungus parasite, then the presence of this parasite in the plant's environment

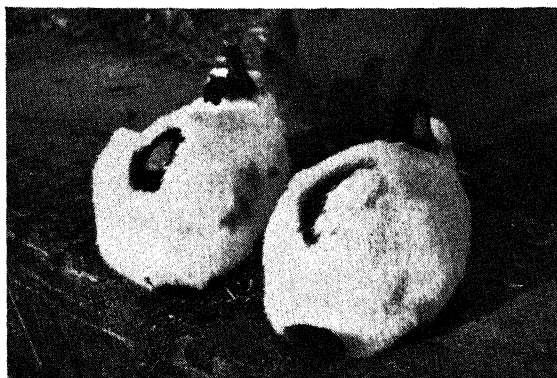


FIG. 3. The effect of temperature on hair color in rabbits. The white hair on a small area of the backs of these Himalayan rabbits was pulled out and the animals then placed in a cold room. The hair which later grew in was black. Hair regenerated in a warm temperature is usually white. (From Laura Kaufman, in *Biologia Generalis*.)

is a necessary condition for the appearance of this peculiarity of the plant. This dependence of all hereditary traits on environmental factors is not so obvious in the structural characters among animals and plants which live in a fairly stable and constant environment, but even here it can be shown



FIG. 4. Sun-red corn. The husks of this ear of corn had been removed and replaced by the black mask below. The kernels developing in the dark are colorless; those exposed to light are red. (From Prof. R. A. Emerson, Courtesy of Department of Plant Breeding, Cornell University.)

to be the case as well. When a variety of maize, known as Sun-red (Fig. 4), is grown in the field, red color appears in the leaves, in the outer husks of the ear, and in other parts exposed to the sun. When these parts are

Smith

shielded from the sunlight, however, no red color develops. The Sun-red trait is inherited but requires the presence of sunlight to develop. In rabbits there is a true-breeding variety known as Himalayan (Fig. 3) in which the pink eyes and the pattern of black ears, feet, and tail and white body are transmitted faithfully to the descendants. If fur is plucked from the white parts and the animal is placed in the cold while the fur is growing in again, the new fur comes in not white but black. On the other hand, if fur is plucked from the black parts and the part is kept warm (by a bandage, for example), the new hair comes in not black but white. It appears at first that the black-and-white pattern is itself inherited, but the experiment shows that what is really inherited is the ability of certain parts to form pigment or not to form it, depending on the particular temperature which obtains in that part at a specific time. More generally, any character is a result both of heredity and of environment.

Genotype and Phenotype. The fact that the individual may express its inherited constitution in a variety of ways, depending on its environment, its stage of development, its age, its relations to other organisms, and similar conditions, indicates the need for a concise way of designating this fundamental peculiarity of living things. The Danish geneticist Johannsen was the first to recognize this clearly, and in 1911 he proposed the terms *genotype* to designate the inherited constitution, the sum total of hereditary factors which an individual receives from its parents, and *phenotype* to designate the appearance of the individual, the sum total of its expressed peculiarities of form, size, color, behavior, both external and internal, gross and microscopic.

We recognize different human individuals or other animals or plants by their phenotypes. The phenotype of an individual obviously consists of many aspects, some of which, such as hair color and quantity, change with age, while others, like his blood group, are constant from before birth to death.

The genotype, on the other hand, cannot be observed directly; it can only be inferred from the ancestry or the progeny of the individual. Some men with brown eyes carry a gene for blue eyes; others do not; but these two genotypes cannot be distinguished by inspection of their eyes. Because of the occurrence of dominance (cf. p. 35) two genotypes may have the same phenotype; and because of the effects of different environments on the same genotype, one genotype may produce a variety of phenotypes.

The genotype is what is inherited; it is what causes offspring to resemble parents. The genotype is stable throughout the life of the individual, except for the rare changes known as mutations. It is the genotype which is responsible for the transformation of materials taken from the environment into an individual which is a more or less faithful copy of its parents.

The genotype directs the development of the plant or animal and, in its interaction with the environment, produces its phenotype. It can be said, therefore, that *the phenotype of an organism is always a result of the interaction of a genotype with an environment.* Thus a human individual, with

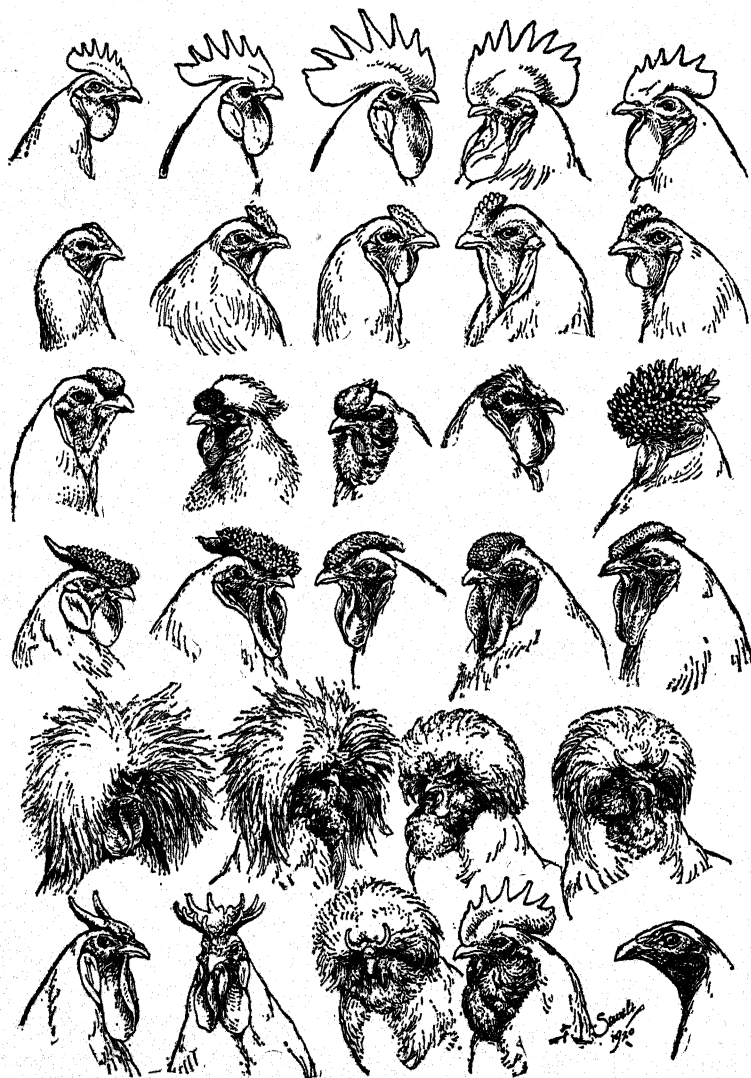


FIG. 5. Variation in the head appendages of male fowls. Each form is typical of a pure breed or variety of domestic fowls. Upper row, types of "single" combs; second row, types of "pea" combs; third row, "walnut" combs; fourth row, "rose" combs; fifth and sixth rows, crests, muffs, Y-type combs, and (lower right) combless. (From Robinson.)

his physical and mental traits, is the product of growth and development brought about by a genotype in a certain succession of environments, and the same is true of organisms generally. Different genotypes in the same environment may produce a variety of more or less different phenotypes.

Variations Due to Heredity. It is evident that genotypic differences are responsible for much of the diversity which is observed among living beings. The same soil and the same climate furnish the environments in which acorns give rise to oaks and grass seeds develop into grasses. The

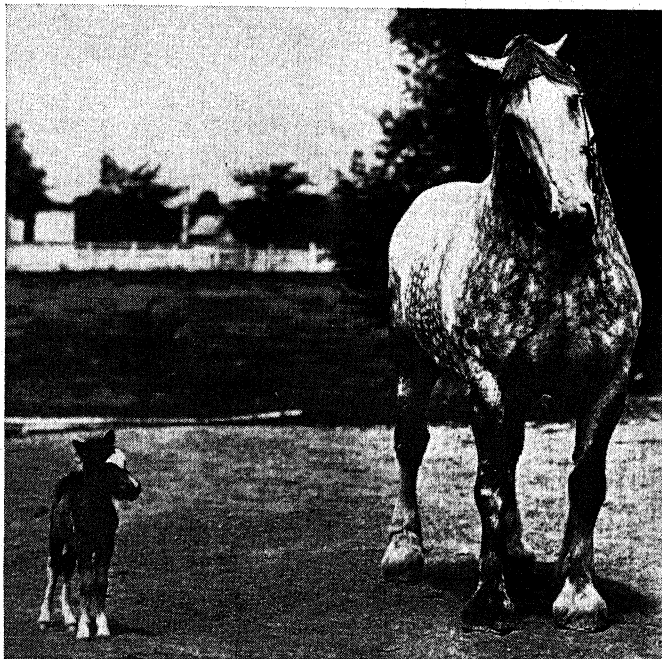


FIG. 6. Extreme size variations in horses. The Clydesdale stallion Kuroki, weight 2,200 pounds, and a Shetland pony foal, weight 21 pounds. (Courtesy of Iowa State College.)

same food makes a puppy grow into a dog and a kitten into a cat. The genotypes of representatives of different species are always different, but even individuals belonging to the same species may have different heredities. Since, however, some of the variations observed among representatives of a species are caused by environmental influences, caution must be exercised in order not to ascribe genotypic variations to environment, and vice versa. The safest way to distinguish between genotypic and environmental variations is by performing experiments in which different individuals or the offspring of different individuals are raised under constant observation in as uniform an environment as possible. Differences between

individuals which have developed in similar environments can be ascribed to differences in genotypes. The converse of this is, however, not always true. Absence of perceptible differences among individuals raised in the same environment does not prove that their genotypes are identical, since not all hereditary differences produce phenotypic differences (*cf.* Dominance, Chap. II).

Figures 5 and 6 represent some varieties of poultry and of horses. The differences between these variations are mainly genotypic. Very instructive experiments on genotypic and environmental variations among plants have been made by investigators beginning in the nineteenth century with Bonnier, who divided the same plant and grew the parts in different environments. Recent observations in California are especially clear. It has been known for a long time that representatives of a plant species which grow in different habitats, especially at different elevations in mountains, may be very different in phenotype. For example, the yarrow, *Achillea lanulosa*, which grows wild at about sea level on the coast of California (the leftmost plant in the lower row in Fig. 7), has a rather tall stem and large leaves and takes about 200 days to reach the flowering stage. At about 4,000 to 5,000 feet above sea level in the Sierra Nevada a somewhat shorter and more slender form is found, which requires only 50 to 60 days to reach the flowering in its native habitat (the second plant from the left in the middle row in Fig. 7). Finally, at about 10,000 feet elevation in the Alpine Zone of the Sierra Nevada there grows a dwarf plant, which in its native habitat takes about 55 days to flower (the second plant from the right in the upper row in Fig. 7). The environments in the native habitats of these plants are very different indeed: at sea level winters are so mild that the plants have little or no winter dormancy, at mid-elevations winters are cold but summers are rather long and warm, while in the Alpine Zone winters are long and severe and summers very short and cool. To what extent, then, can the differences between the yarrow plants that are native in these habitats be ascribed to genotype and to environment? To answer this question, yarrow plants collected in several localities were each cut into three parts, and these parts were replanted in three experimental gardens: at about sea level (Stanford), at 4,600 feet (Mather), and at 10,000 feet (Timberline). The division of the same plant ensures that each part will have the same heredity. The results of this experiment are shown in Fig. 7. The horizontal rows show the plants native in different habitats when grown together in the same experimental garden. The differences between the plants in the horizontal rows are, accordingly, genotypic. The vertical rows show how the same plant behaves in different environments; these differences are environmental. The differences in these plants are thus due to the ways in which specific genotypes respond to specific environments.

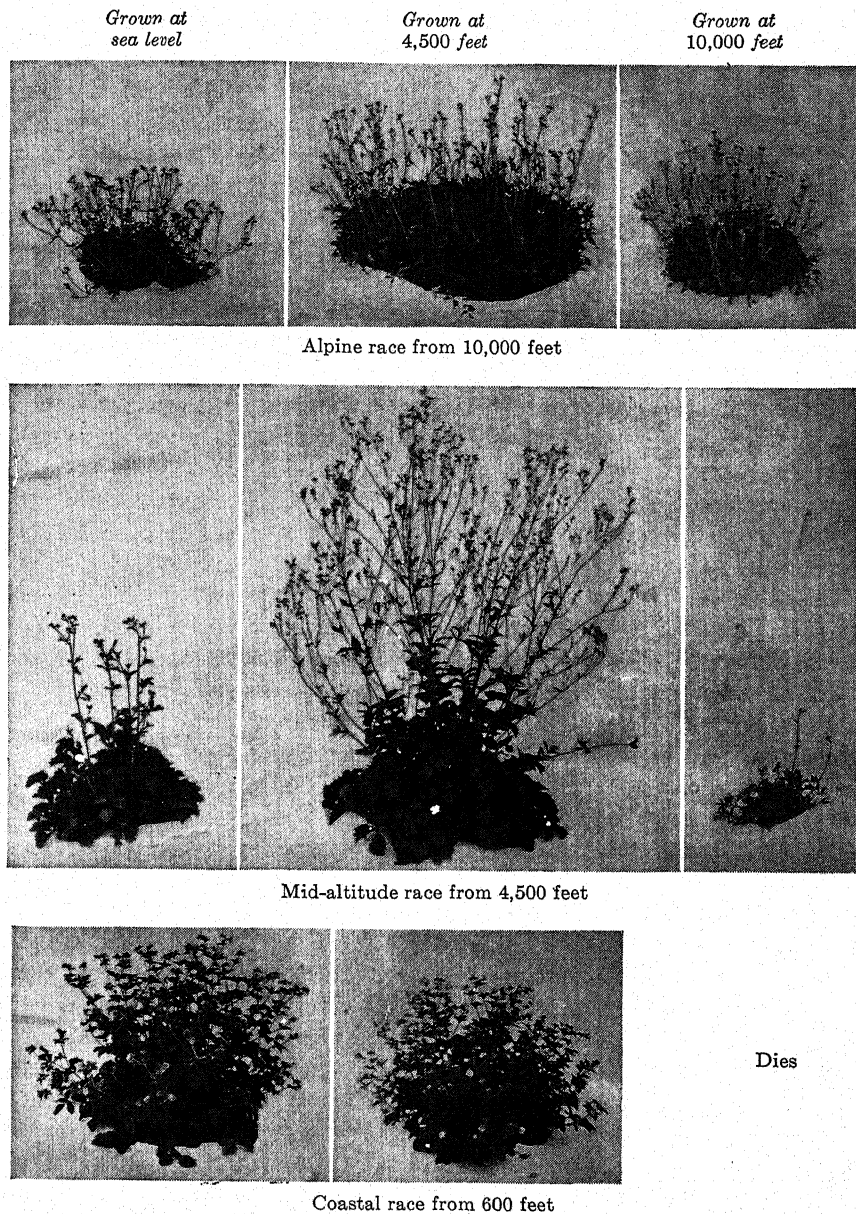


FIG. 7. Plants of three altitudinal races of the yarrow, *Achillea lanuloso*, divided and grown in different environments. The differences among the plants in the vertical columns are genotypic, due to selection and adaptation of their ancestors to different altitudes; the differences among the plants in the horizontal rows represent responses of the same genotype to different environments. (From Clausen, Keck, and Hiesey.)

Of course, many genotypic differences result in phenotypic distinctions that are far subtler than those shown in Figs. 5 and 6. The work of breeders of agricultural plants and animals consists essentially in obtaining genotypic variants which, when raised under farm conditions, give greater yields of grain, fiber, milk, meat, wool, or other products useful to man. Even slight improvements in yield are desirable if these improvements are genotypic and thus may be expected to recur again and again in the progeny of the improved variety. However, yields are influenced not only by genotype but by such environmental factors as the quality of the soil, amount of moisture, heat, and light, and the quality and quantity of the fertilizer or food supplied. It is evidently important to know whether a difference in yield observed between two or several samples planted in experimental plots is predominantly genotypic or environmental. Out of this practical need, there has grown a whole branch of agricultural science which devises experimental procedures whereby the relative influence of genotypic and environmental factors can be ascertained. In brief, these procedures consist in making replicated plantings which reduce the environmental disturbances. The data are treated with the aid of statistical methods devised for this purpose.

Variations Due to Environment. If individuals with similar genotypes are raised in different environments, the phenotypic differences that may be observed among them can be ascribed to environmental influences. In experiments of this sort it is evidently important to be sure that the experimental animals or plants are indeed genotypically uniform. It is easy to provide this condition in some species but very difficult in others.

The situation is simplest in organisms which reproduce asexually—by fission, by buds, bulbs, tubers, runners, etc. The progeny descended from a single individual by asexual means is uniform in genotype; such a progeny is called a *clone*. Genotypic diversity may arise within a clone only by mutation, but mutations are usually rare (see Chap. XI). Members of a clone are often remarkably similar in appearance when grown in a uniform environment, but they may become sharply diversified if exposed to different environments (Fig. 7). The plants grown from cuttings obtained from a single individual constitute a clone, yet their phenotypes may be less similar in different environments than the phenotypes of representatives of different clones in the same environment.

Individuals with similar genotypes also arise under certain types of sexual reproduction. Most plants and some animals are *hermaphrodites*, that is, they carry female and male sex organs, and the same individual produces female and male gametes. In some hermaphrodites, zygotes may be formed by a union of gametes developed in the same body; this method of reproduction is known as *self-fertilization*. Self-fertilization is the rule

in some plant species, for example, in most wheat varieties. Among animals, it is rare; however, it does occur, for example, in some fresh-water snails. The progeny of a single individual obtained by self-fertilization is called a *pure line*. The genotypic uniformity of members of a pure line is likely to be greater than that in a progeny obtained by cross-fertilization.

Johannsen's classical experiments on interrelations of heredity and environment were made on pure lines of the bean plant. Johannsen took several seeds from a commercial variety of beans, grew plants from these seeds, self-fertilized the flowers, and collected seeds of the next generation separately from each plant. By repeating this procedure in several generations, he obtained several pure lines, which he raised in a reasonably uniform environment. Weighing of the seeds disclosed that the average weights were different in the different pure lines. These average differences are due to heredity. But within a pure line some seeds were also heavier or lighter than others. Are these differences between members of a pure line genotypic or environmental? To answer this question, Johannsen selected some of the lighter and some of the heavier seeds within each pure line, and grew progenies from them separately. As shown in Table I, the average seed weight in these progenies was essentially the same. The differences between seeds within a pure line are, therefore, phenotypic. They are caused by uncontrolled variations in the environment. The differences between seeds within a commercial variety of beans from which the pure lines have been isolated were, however, of mixed origin: partly genotypic and partly environmental.

TABLE I. THE RESULTS OF SELECTING THE LARGEST AND THE SMALLEST SEEDS IN TWO LINES OF BEANS DESCENDED FROM A SINGLE PLANT (*Data from Johannsen*)

Generation	Average weight of selected parent seeds		Average weight of progeny seeds	
	Lighter seeds	Heavier seeds	From lighter parent	From heavier parent
1	60	70	63	65
2	55	80	75	71
3	50	87	55	57
4	43	73	64	64
5	46	84	74	73
6	56	81	69	68

In many organisms neither asexual reproduction nor self-fertilization is possible, and consequently neither clones nor pure lines can be obtained. In such organisms, one resorts to inbreeding, that is, to mating of close relatives, such as brothers and sisters. After some generations of in-

breeding, *inbred lines* are obtained, in which the genotypic uniformity is greater than it was in the initial, or crossbred, populations. Inbred lines are important both in scientific experiments and in agricultural practice. Thus, inbred lines of maize are the foundation of the high-yielding "hybrid" corn. One can measure the effects of environmental factors, such as amount and kind of fertilizer and type of soil, on crop plants by growing



FIG. 8. The effect of amount of light on flowering time in the chrysanthemum. Plants of the variety Cordova. At the left a plant which received no extra light; each of the three plants at the right received one hour extra light at midnight from a 100-watt bulb, the leftmost from September 25, the center one from October 9, the rightmost from October 23. Extra light has delayed the flowering time. (Courtesy U. S. Department of Agriculture.)

members of the same inbred line under different experimental conditions. Only in this way can the genotype be held constant while environmental effects upon the phenotype are allowed to vary. Similarly, in laboratory animals such as mice, rats, or guinea pigs, the existence of inbred lines with a high degree of genotypic uniformity is an essential prerequisite for the measurement of environmental factors in nutrition, sensitivity to disease, effects of drugs, and many others.

Some environmental effects are so striking that no high-precision experiments are needed to detect them. Thus, it is easy to observe the striking differences in height, number of leaves, and yield of fruit or seed which distinguish plants grown in poor soil from others of the same variety which have grown in rich soil. The effect of nutrition may be sharp and specific as in sweet potatoes, which when grown in soil rich in potassium are round and fleshy but when deprived of this element produce long and spindling roots. In the same way, the quality and quantity of food determine to a



FIG. 9. Monozygous or identical twins. Lois and Louise were separated at birth and grew up in different households. They were reunited as students at Baylor University. (Courtesy of Prof. H. H. Newman.)

considerable degree the size and productivity of animals. The precise ration fed to a steer, a cow, or a laying hen makes a great deal of difference in the number of pounds of beef, quarts of milk, or dozens of eggs which are produced. So powerful is the effect of the environment on characters of this sort (which are obviously very important ones economically) that most of our agricultural practices are concerned with such a manipulation of the environment—whether of fertilizers, water, temperature, food, or other factors—that the most desirable variations shall be induced. The amount and kind of light determine in plants the degree of development of the essential green pigment, chlorophyll, and the time of flowering and fruiting

(Fig. 8); while in many animals sunlight is responsible for the production of specific essential factors such as vitamin D. The separate factors of nutrition may have very specific effects, as, for example, the appearance of rickets or of the striking changes brought about by scurvy or by beriberi, where some single vitamin is deficient in the diet. The practice of medicine, too, is chiefly concerned with modifying the animal organism through its environment.

Heredity and Environment in Man. The problem of the relative importance of heredity ("nature") and environment ("nurture") in the development of human bodily traits and of intellectual and emotional faculties has not only biological but also sociological and philosophical implications. No final and generally convincing solution of this problem has as yet emerged. The obvious difficulty has been that no controlled experiments with human beings can be conducted. Traits such as alcoholism, criminality, poverty, nomadism, or their opposites are frequently observed to recur in some families generation after generation and because of this are often ascribed to heredity. This conclusion does not follow, because the facts can often be at least as well accounted for by assuming that those traits are environmental. It is evident, for example, that a child growing up in a family in which criminal conduct is regarded with favor is more likely to fall into delinquency than a child in a family in which honesty and uprightness are admired. Similarly, social eminence is more easily attained in families of wealth and refinement than in surroundings of poverty and ignorance.

One of the best opportunities for assessing the relative effects of heredity and environment upon specific traits in man is afforded by the study of identical twins. About once in every 80 births two babies are born, and in about a quarter of these the twins are identical, that is, they develop from a single fertilized egg. *Identical*, or *monozygous*, twins (Fig. 9) are two members of a clone, and they have the same heredity. The remaining twins, known as *fraternal*, or *dizygous*, are merely two individuals which arise from two separate eggs which happened to be fertilized at the same time (Fig. 10). The genotypes of fraternal twins are no more alike than those of brothers and sisters not born simultaneously. Differences that may be observed between identical twins when they grow and develop are to be attributed to environmental factors; those between fraternal twins are partly genotypic and partly environmental.

The strikingly greater physical resemblance between identical twins than between fraternal twins indicates that this resemblance is due to their identical genes rather than to similar environments, which fraternal twins also share. Identicals are always alike in sex, blood groups, hair and eye color, and many other physical traits, while fraternal twins may differ in any of them.

This is good evidence that heredity is the deciding factor in respect to these traits. Even more significant are observations on identical twins which have been separated at an early age and reared in different environments. Since identical twins reared apart retain their similarities in most physical features, these features are shown to be relatively independent of the type of environmental influences encountered after birth. The situation is



FIG. 10. Dizygous or fraternal twins. Mrs. Olivia Strong with her twins, Eddie Ray, an albino, and Lucy May, normally colored. (*International News Service.*)

quite different with respect to intelligence and scholastic success and generally with respect to mental and emotional traits. Identical twins reared apart are often quite appreciably different in such traits, although still somewhat less so than is the case with fraternal twins. Psychic traits seem to be, accordingly, more susceptible of environmental modification than are physical traits. The relation between heredity and environment is not one general question but a series of specific questions about specific traits. We owe much of the systematic study of twins to Newman and

his coworkers, and their books (as listed at the end of the chapter) should be consulted for the evidence and a fuller discussion.

The Reaction Range. We have seen that, when individuals that have the same genotype develop in different environments, they exhibit more or less different phenotypes. Every genotype reacts with its environment in its own special manner; but if the same genotype has somewhat different materials to work on, the phenotypes may be appreciably different. *What a genotype determines is the reactions, the responses, of the organism to the environment.* Since the variety of environments in the world is very great, many different phenotypes may develop from the same genotype. These potentially possible or actually realized phenotypes constitute the *range of reaction* of a given genotype. In practice, one is never certain that the entire range of reaction of any genotype is known, because possible environments are too numerous to make experimental determination of the reactions of a given genotype to all of them practicable. It is, however, evident that, the more thorough becomes our knowledge of the reaction ranges of human genotypes and of those of agricultural plants and animals, the greater will be our ability to control them according to our will. When medical men and hygienists study the effects of various treatments, drugs, and living conditions on human bodies or when educators experiment with various methods of teaching, their purpose is to increase our understanding of the ranges of reaction of human beings. When agriculturalists investigate the influence of soils, fertilizers, and methods of feeding and caring for crop plants or domestic animals, they endeavor to discover what possibilities useful for human welfare may exist in the ranges of reaction of the cultivated plants and animals. Any phenotype, normal or pathological, which arises under the influence of any environment, natural or created artificially by man, is evidently within the range of reaction of the genotype which produces it.

One of the most significant facts about the ranges of reaction is that different traits of the same organism exhibit different degrees of plasticity to environmental influences.* We have seen that identical twins in man always have the same blood groups. The blood group to which a person belongs is already expressed in the embryo, and it remains unchanged until death, regardless of the state of health or of the climate or other conditions under which the person may be living. The blood group is rigidly determined by heredity. The range of reaction of human genotypes with respect to skin color is much broader than with respect to blood groups. Albinos form no pigment in the skin, and the skin color of people of European descent is lighter than in natives of central Africa. Yet it is well known that the skin color in most people is also influenced by exposure of the skin to ultraviolet radiation or to sunlight. Skin color is surely inherited, but it

can also be modified by environmental influences. "Intelligence," as measured by the so-called IQ (intelligence quotient), is influenced by heredity (as shown by studies on identical twins and other data); yet education and training can alter the IQ within such wide limits that an individual who starts with an IQ lower than another individual may eventually catch up and overtake him. Manners and behavior are doubtless determined very largely by cultural influences of the society of which the individual is a member. Yet it is possible to show that heredity may also have an influence on human behavior. For example, certain forms of diabetes, a disorder which disturbs normal carbohydrate metabolism and causes excretion of sugars in the urine, are hereditary. A person afflicted with diabetes must exercise a strict choice of diets and must receive at regular intervals injections of insulin, a substance which enables the metabolism to proceed more or less normally despite possession of diabetic heredity. It is not at all strange that the personality and behavior of a diabetic acquire certain peculiarities caused by these necessities and, hence, by heredity.

All possible intermediate conditions exist between a rigid genotypic determination of traits, as exemplified by the blood groups, and an environmental plasticity which eclipses and conceals genotypic influences, as seen in the case of human behavior. Furthermore, the degree of environmental plasticity of a trait in plants, animals, or human beings depends upon the degree to which we understand and control specific environmental variables. Diabetes, a disease which depends upon the genotype, may now be controlled by insulin. We may even be phenotypically normal in the presence of the virus of smallpox, a disease to which we are genotypically susceptible, provided that we are immunized against this virus. Also, some of the fungus parasites of wheat may now be neutralized by keeping the infective genotype (the specific type of rust) out of contact with the reactive genotype (a specific variety of wheat).

Sometimes the environment may produce in normal individuals a replica, or *phenocopy*, of a hereditary variant which has already been known to be due to a changed genotype. Thus the phenotype yellow body in the vinegar fly *Drosophila* may be due either to a gene for yellow or to the inclusion of silver nitrate in the food on which larvae with normal genes have been reared.

What we inherit thus is the mode of response to the environment. This is nowhere better illustrated than in the Himalayan albino rabbit (Fig. 3). Clearly it is not the color pattern which is inherited but the form of response to the environment, in this case indicated by the differing response of the various body regions to temperature. The Himalayan gene sets the stage for this response.

The variety of response which the same hereditary constitution may

make is the hopeful basis on which the amelioration of the environment by human and veterinary medicine and many agricultural and social practices rest. It also explains why fundamental changes in the range of reaction of a population to an optimum environment require a change in its genotypic constitution by means of genotypic variation and selection.

Inheritance of Acquired Characters. Biologists and nonbiologists alike are familiar with two sets of facts about variation and heredity. First, organisms are modified by environment. Sustained use or exercise of an organ, such as a muscle, or of a faculty, such as skill in performing certain kinds of work, generally strengthens and develops the organ or the faculty.

Disuse and lack of exercise weaken or reduce organs and faculties. Second, offspring tend to resemble their parents. It is tempting to connect these two sets of facts and to conclude that modifications induced in the parents by the environment (*acquired characters*) will be transmitted to and inherited by the offspring. The hypothesis that acquired characters may be inherited seems so reasonable that it was accepted as true by most biologists up to and including Darwin, Spencer, and their contemporaries, without careful test or verification.



FIG. 11. Jean Baptiste Lamarck (1744–1829).
(After Locy.)

Lamarck (Fig. 11), the great French biologist (1744–1829) who was the father of the first modern theory of biological evolution,

considered inheritance of acquired characters to be the most important, if not the sole, mechanism of evolutionary change. The type of evolutionary thought which accepts this view is known as *Lamarckism*. According to Lamarck, variations are induced in organisms in response to an urgent need and striving on the part of individuals and through use or disuse of organs. The modifications so called into being he regarded as heritable. Indeed, to Lamarck, all variations are acquired, and all variations are heritable. Darwin considered natural selection to be the directing agent of the evolutionary process. Natural selection operates with hereditary variants, most of which were regarded by Darwin as spon-

taneous changes of unknown origin. Nevertheless, Darwin fully accepted the inheritance of acquired characters as an important, though subsidiary, factor in evolution.

August Weismann (1834–1914) (Fig. 12), perhaps the most important forerunner of modern genetics and the initiator of the so-called *neo-Darwinian* school of evolutionary thought, questioned whether acquired characters are actually inherited, made the first controlled experiments which

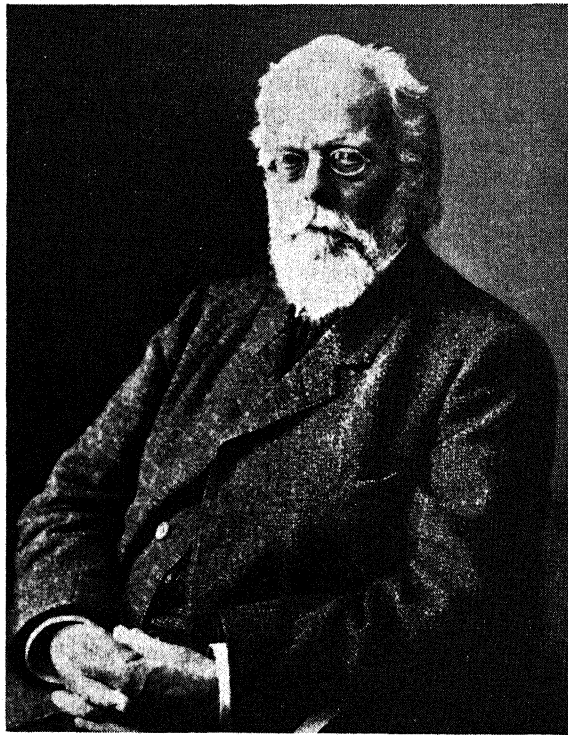


FIG. 12. August Weismann (1834–1914). (Courtesy of Genetics.)

led to negative results, and developed the *germ-plasm* theory, which constituted a large step forward in understanding heredity. The reproductive tissue of animals (the germ cells and cells from which they arise) he regarded as distinct and separate from the other tissues of the body (*somatoplasm*). Changes induced by the environment in the somatoplasm are not transferred to the germ plasm, which is the sole channel through which heredity is transmitted to the offspring. Acquired characters could not, therefore, become inherited. Weismann's experimental evidence consisted in cutting off the tails of mice for a series of generations and observing that the progeny

still had tails of normal length. This procedure seems exceedingly crude, but it is well to remember that such hereditary variants as hornlessness and taillessness in animals have been frequently thought by older authors to have arisen from the practice of dehorning or of docking the tail continued over many generations.

Although the conception of a distinct germ plasm is useful and aids in explaining many facts and in planning experiments with higher animals, it is undoubtedly of limited application. It does not apply at all in the lower animals, where no practical distinction between soma and germ can be made, or in plants, where many or all parts of the plant body may give rise to germ cells or to new individuals without the intervention of a sexual process. Even in the higher animals it is possible that the gametes may arise from tissue which is not fundamentally different from that which produces other parts of the body and that the gonads are not completely insulated from those forces which effect changes in the body tissues.

The formulation of the concepts of genotype and phenotype by Johannsen and his experiments on pure lines of bean plants (see p. 17) caused the belief in inheritance of acquired characters to be discarded by almost everybody familiar with genetics. It will be remembered that the progenies from large and from small beans in a pure line are of the same average size (Table I). Among members of a pure line, the variation in size of the beans is, however, an acquired character, and it would be expected to influence the size of the beans in the progeny, if such characters were inherited. In general, any modification of the phenotype, by whatever means induced, constitutes a response determined by the genotype of the individual concerned to a certain environment. Any acquired character is, therefore, within the range of reaction of the organism.

The possibility that acquired characters may be inherited ceases to be plausible as soon as one frees oneself from the oversimplified notion that what is inherited are "characters," traits, or organs of the adult, and, instead, conceives of heredity as a series of dynamic responses of the organism to the environment in the process of development. It can be shown that the genotype that determines these responses is not changed thereby. This certainly does not mean that the genotype is not changeable, or, for that matter, cannot be changed by environmental influences. In fact, genotypic changes (mutations) do occur from time to time probably in all organisms, and they can be induced or at least speeded up by such environmental agencies as X rays, temperature, and chemical treatments (see Chap. XI). But the fundamental property of the genotype is precisely that it reproduces itself, by imposing its own pattern on the nutriment from the environment. In the last analysis, the development of the body can be regarded as a kind of by-product in the process of self-reproduction

of the genotype, which may be the essence of life. Samuel Butler put this in a nutshell by saying "the hen is the egg's way of making another egg."

It may be stated that the experimental evidence now available in biology indicates that acquired characters are probably never inherited. This negative evidence is very considerable, showing that the effects of poisons, alcohol, variations due to amounts or kinds of food or to temperature or light, and effects of acclimatization and domestication are not transmitted *as such* to descendants. The few experiments leading to opposite conclusions are of doubtful validity and do not in any case amount to proof. The few remaining adherents of Lamarckism usually admit the lack of experimental support of their views but insist that inheritance of acquired characters must nevertheless be assumed to occur in order to explain evolution. In later chapters it will be shown that the phenomena of evolution do not necessitate any resort to such unverified hypotheses.

The folklore of many peoples includes some erroneous notions about heredity, apart from the belief in inheritance of acquired characters. Assertions are sometimes made that, in man and other mammals, the developing embryos may somehow acquire a resemblance to objects seen by the pregnant mother, especially if these objects evoke in the mother a strong emotional reaction ("maternal impressions"). Or it is believed that a male not only influences the child of which he is the father but transmits some of his traits to children of the following pregnancies in the same mother produced by other males (telegony). It is also believed by some that individuals in vigorous health influence the characteristics of their progeny more strongly than do parents in enfeebled condition. These beliefs, in spite of their persistence in folklore and even among some modern agriculturalists, have no foundation in fact.

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PROBLEMS

1. Other things being equal, would you expect greater variability among organisms reproducing asexually or among bisexual species?
2. What has been the effect of human variability on the development of human societies?
3. What explanation have you for the present great variability of the human race? Why would you expect the population of the United States to be more variable than that of most European countries?
4. *Drosophila* larvae which have plenty of food and grow at moderately low temperatures develop into larger flies than do similar larvae which grow at high temperatures and on semistarvation diet. The fly species *Drosophila miranda* is larger than *Drosophila pseudoobscura* if the larvae develop at the same temperature and with plenty of food. But *Drosophila miranda* developed at high temperature and with little food is smaller in body size than *Drosophila pseudoobscura* grown at lower temperature and with abundant food. Is the body size a hereditary trait in these flies?
5. What experimental procedure can you suggest to test whether two strains of *Drosophila* flies (or of any other organism) are genotypically different in body size?
6. Normal ("wild type") strains of *Drosophila melanogaster* have grayish-brown bodies if developed on food media free of silver salts, but have yellow bodies if certain silver salts are added to the food on which the larvae develop. Strains of the mutant *yellow* in the same species have yellow body color regardless of whether they develop on food with or without silver salts. Suppose you have a yellow fly and the food on which it has developed is unknown. How would you find out whether this yellow fly is a yellow mutant or a yellow phenocopy?
7. The practice of docking (cutting off) the tail in puppies is commonly followed in fox terriers and certain other dog breeds. In these (and in other) breeds some puppies are born with short or with no tails, and this taillessness is transmitted to the offspring. Is this situation valid evidence of inheritance of an acquired trait? Explain.
8. What evidence would you require to demonstrate that docking the tails in puppies changes their heredity?

9. The hair color of an individual may be brown in youth, black at maturity, and white in old age. What color would you call his hair in a study of inheritance of hair color in his family? In investigating the inheritance of hair color, what precaution, therefore, should be exercised?

10. Some races of teasel have spirally twisted stalks if grown in rich soil but normal straight ones if grown in poor soil. How does a normal plant from such a race differ genetically from a normal plant of a race which never shows twisting?

11. Measles and chicken pox killed thousands of natives of the South Sea Islands at the first coming of the white men, but these diseases are rarely fatal to Europeans. Explain.

12. The Napoleonic wars are said to have reduced somewhat the average stature of the French people. To what might such a result be due?

13. The chestnut bark fungus, introduced some years ago into the United States, has exterminated all the native American chestnut trees over a wide area. In China, its native home, the species of chestnut are almost immune to its attack. How do you explain this difference between American and Chinese chestnut trees?

14. How could you determine whether a given case of variation is due to environmental or genetic influences?

15. What difficulties are encountered in studying the inheritance of such characteristics as size, yield, and intelligence?

16. How can a study of twins be used in helping to assess the relative influences of heredity and environment in susceptibility to tuberculosis in man?

17. Give your interpretation of the following data:¹

	Numbers of pairs of twins	
	Both members convicted of crime	One member only convicted of crime
Identical twins	45	21
Fraternal twins (same sex)	32	52

18. What advantages and what disadvantages to human society would result from the inheritance of acquired characters?

19. How could you explain the possession by animals of highly developed instincts which the individual itself has had no opportunity of acquiring, assuming (1) that acquired characters are inherited; (2) that they are not?

¹ Jour. Heredity, October, 1936.

20. If members of a white-skinned race are exposed to bright sunlight, their skin is darkened, or "tanned." Races native to regions of bright sunlight, like Negroes in the tropics, are genetically dark-skinned. How would Lamarck explain the dark skin of such races? How would you?

21. How can you tell whether a new trait is a mutation or the result of complex segregation following a cross?

22. If selection within a pure line is ineffective, how do you think that different pure lines, in beans for example, originated?

CHAPTER II

MENDEL'S PRINCIPLE OF SEGREGATION

The chief conclusion from the previous chapter is that the appearance, structure, physiology, and behavior of any plant or animal, in short its phenotype, are determined by the interaction of its genotype with the environment. It is now logical to inquire into the nature and composition of the genotype, which plays such a fundamental role in the phenomena of life. It is noteworthy that the genotypes of all organisms, from man and higher animals and plants down to bacteria, consist of genes. Because of the apparently universal applicability of the gene theory, the gene may be regarded as the fundamental unit of life. Genes in biology are to this extent comparable with other structural units of matter such as molecules and atoms.

Historically the first and still the most conclusive evidence of the existence of genes comes from the phenomena of *segregation* of traits observed in the offspring of hybrids between individuals or strains which differ in some recognizable respect. The principle of segregation was formulated by Gregor Mendel in 1865.

Mendel and His Method. This principle, which is now recognized as among the most important in biology, was formulated under such peculiar circumstances that the scientific world failed to recognize or to appreciate it until after a lapse of forty years. In the first place, Gregor Mendel was not primarily a biologist but a monk in the Augustinian monastery at Brunn, Austria (now Brno, Czechoslovakia) (Fig. 13). He had come as a poor boy to the monastery, was ordained priest in 1847, and in 1851 was sent by his order to study natural science at the university in Vienna. He was not a brilliant scholar in physics or mathematics, but it is quite clear from his published work that he had an unusually clear and logical mind. He returned to Brunn as teacher of science in 1853, and in 1857 he began to collect and to observe the numerous varieties of the garden pea which seedsmen offered for sale. These varieties differed in height, flower color, seed color and other ways, and they seemed to Mendel to provide suitable material for answering a simple but important question which no botanist up to his time had even formulated clearly, to say nothing of obtaining an answer. After observations and experiments carried on in the monastery gardens for seven years, he obtained the answer he had sought and pre-

sented the results of his hybridization experiments together with the generalizations which we now know as Mendel's laws at two meetings of the Natural History Society of Brunn (Naturforschender Verein) in the spring of 1865. The results and the theory were printed in the annual proceedings of the society, which appeared and were distributed to libraries in Europe and America in 1866. It is safe to say that no one who heard Mendel's paper and no one who read it in the nineteenth century appreciated its significance, for it lay neglected until 1900, when suddenly the law of segre-



FIG. 13. The garden in the Königinkloster in Brunn in which Mendel carried out his experiments with peas, 1856-1864. (From a photograph by Prof. Hugo Iltis.)

gation was rediscovered almost simultaneously by three different investigators, who had obtained results like Mendel's. These three—De Vries in Holland, Correns in Germany, and von Tschermak in Austria—found Mendel's forgotten paper and proclaimed its importance. Immediately Mendel's conclusions began to be confirmed and extended by experiments carried on in various parts of the world on many kinds of animals and plants.

Meantime, Mendel, who realized the importance of what he had done, turned at first to experiments with other plants and with bees and to mete-

orological observations but gradually became more and more concerned with the administrative work of the monastery, of which he had become abbot in 1868. The last years of his life were embittered by the struggles in behalf of the monastery against the tax power of the state and, one may imagine, by the frustration of a scientific mind which had been unable to convince or even to interest his contemporaries. He died in 1884 long before his scientific work came into its own.

In order to understand his work we must examine his methods. Since the time of Kölreuter several investigators had made hybridization experiments with plants, and Mendel was familiar with the work of his predecessors. The reason why it remained for Mendel to discover the laws now bearing his name lies in his wise choice of material and methods of study.

Mendel endeavored to avoid the complexities which had troubled the earlier students by simplifying the problem as far as possible. His predecessors had noticed that, when plants of different species or varieties were crossed, great variability appeared in the progeny, as had been apparent also from the results of breeding mongrel animals. However, the early investigators were making observations upon the plant or animal as a whole, studying at once all the traits and structures in which hybrids differed from the parents and from each other. Mendel confined his attention to a single character at a time (Fig. 14), such as flower color. When the behavior of each single trait was established, he then studied two traits together, such as flower color and vine height. Then he counted the members of each type of progeny which resulted from the cross, thus reducing the phenomena of inheritance to a measurable, quantitative, basis.

These innovations of Mendel—counting the different types of offspring, studying single characteristics independently of the whole individual, and keeping accurate pedigree records of the members of successive generations—are simple enough in themselves, but such a thorough application of the experimental method to breeding problems had never been made by any other investigator. The important discoveries which Mendel made were largely due to these new methods, and they have since been the basis of all careful genetic research.

The garden pea proved to be very satisfactory material for experiments on hybridization. Its flowers are so constructed that the reproductive parts are covered by the petals and not exposed to insects. Pollen normally falls on the stigma of the same flower and thus effects self-fertilization. Mendel could open a flower bud and remove the stamens before any pollen had been shed, thus preventing self-pollination. He could then place on the stigma of this "castrated" flower, pollen from the plant which he wished to use as the other parent in a cross. The artificially fertilized flowers were guarded against contamination by pollen of unknown origin

by preventing the access of insects to these flowers. If he wished to determine the kind of progeny which would appear in the second hybrid generation following the cross, he had only to allow the flowers to fertilize themselves normally.

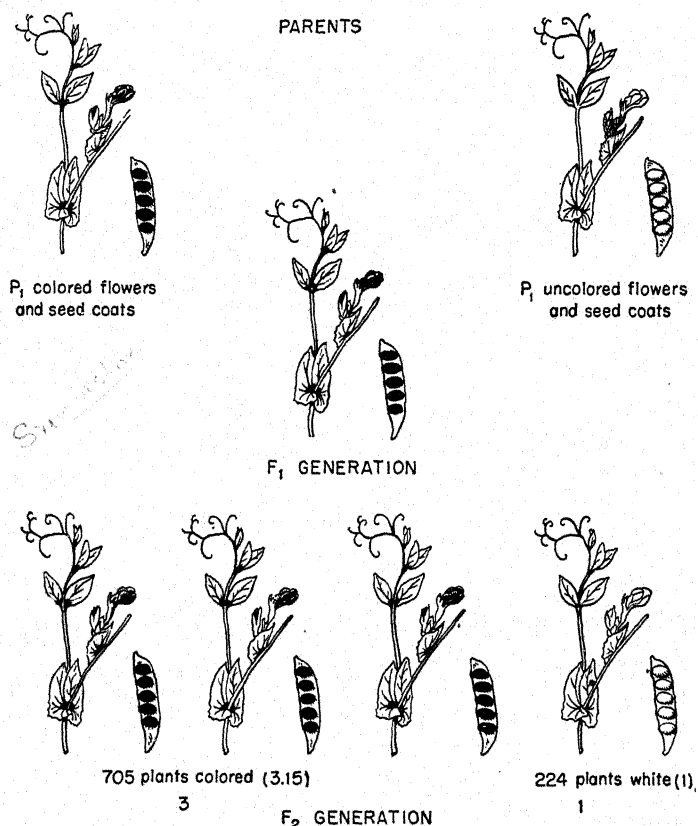


FIG. 14. Outcome of one of Mendel's first experiments. Colored flowers, colored spots in leaf axils, and colored seed coats constitute one "differentiating character" as contrasted with the colorless conditions. This character is inherited as a unit. (After Iltis.)

Mendel's procedure was to cross two plants differing in a pair of contrasted characters, to plant the seed thus obtained, and to observe the appearance of the first hybrid, or "F₁," generation.¹ He then crossed two hybrid plants together (or allowed them to effect self-fertilization) and raised as large a number as possible of second generation, or F₂, offspring. These were found to display more or less variation in the character studied,

¹ The parental generation is technically known as the P₁, the first generation following a cross as the F₁ (first filial generation), the second as the F₂, and so on.

and he accordingly classified them, counting the number of plants possessing each of the contrasted traits.

From a study of such comparatively simple data were formulated hypotheses which have since been so widely verified by experiments with many other plants and animals that they are now clearly established as Mendel's laws of inheritance. These include the two major principles of

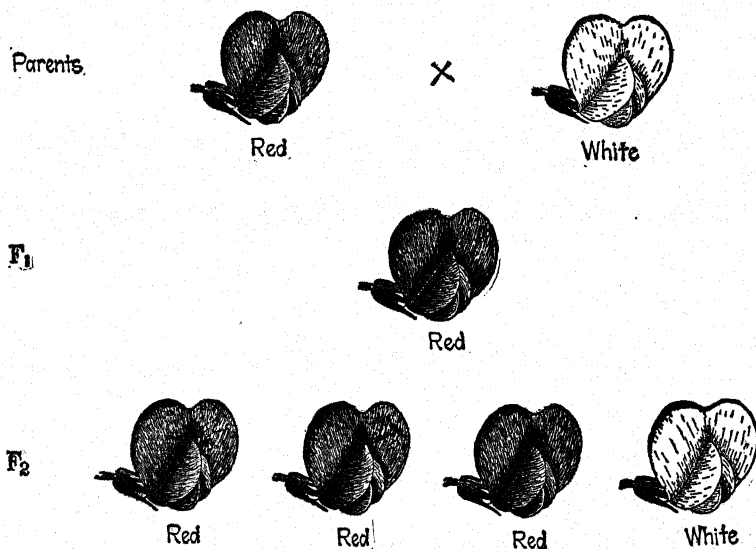


FIG. 15. Cross between a pure red-flowered and a white-flowered pea plant, showing the dominance of red flower color in F_1 . If an F_1 plant is self-fertilized the resulting F_2 generation is $\frac{3}{4}$ red-flowered and $\frac{1}{4}$ white.

segregation and independent assortment, together with a number of less fundamental generalizations.

The accounts of the experiments which Mendel performed, and of the formulation and testing of the hypotheses, are given so clearly and concisely in Mendel's paper that they should be consulted in the original (see Appendix).

Dominance. One of the first facts brought out by Mendel's experiments was that the two members of a given pair of contrasting characters, when brought together in a cross, differ markedly in their ability to express themselves in the resulting hybrid offspring. When he crossed a pure-breeding red-flowered plant with a pure-breeding white-flowered one, for example, the progeny were found to resemble the red-flowered parent (Fig. 15). No white-flowered plants and no intermediates appeared. He knew that whiteness had not really been eliminated, for in the subsequent generation white-flowered plants cropped out again; but in the hybrid itself whiteness

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seemed to be suppressed or to recede from view and redness to dominate. Mendel therefore called such a trait as redness of flowers a *dominant* one and such a trait as whiteness a *recessive* one. All of the seven characters in peas reported by Mendel behaved in this way, one of each pair of contrasting traits appearing to be dominant and the other recessive. Thus the round form of seed was found to be dominant over the wrinkled; the yellow color of the cotyledons over the green; the inflated form of pod over the constricted; the green color of the unripe pods over the yellow; the axillary

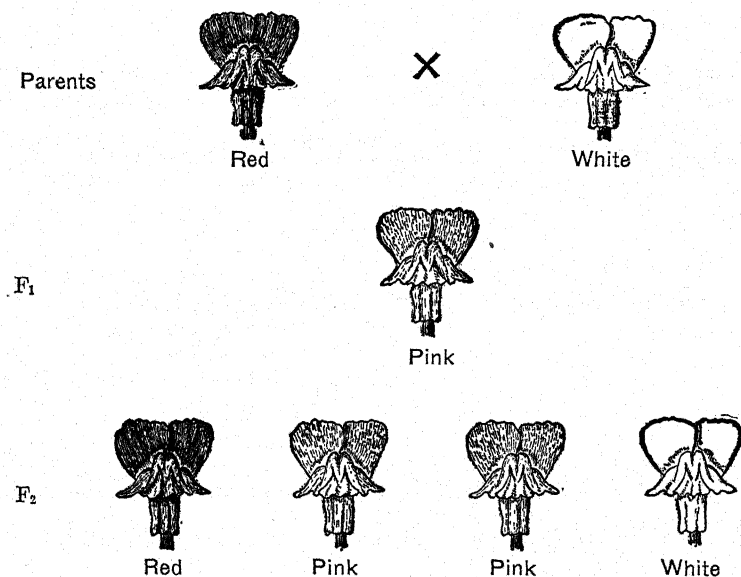


FIG. 16. Cross between a red-flowered and white-flowered snapdragon showing absence of dominance in F₁. If an F₁ plant is self-fertilized the resulting F₂ generation is $\frac{1}{4}$ red, $\frac{1}{2}$ pink, and $\frac{1}{4}$ white.

position of the flowers and pods over the terminal; and the tall vine habit over the dwarf.

Later investigators have also found many characters which show similarly complete or practically complete dominance. In very many other cases, however, dominance is absent, and the hybrid individuals resemble neither parent exactly but are more or less intermediate between the two. In the snapdragon, for example, a crimson plant crossed with a white one gives first-generation hybrids which are all *pink* in flower color (Fig. 16). In the same way a black Andalusian fowl bred with a splashed white one produces offspring which are "blue" in the color of their plumage (Fig. 17); and in Shorthorn cattle the cross of red coat by white gives offspring which are

"roan," their coats consisting of a mixture of red and white hairs. In other instances the hybrid offspring may resemble one parent much more closely than they do the other but may not resemble it exactly, so that dominance

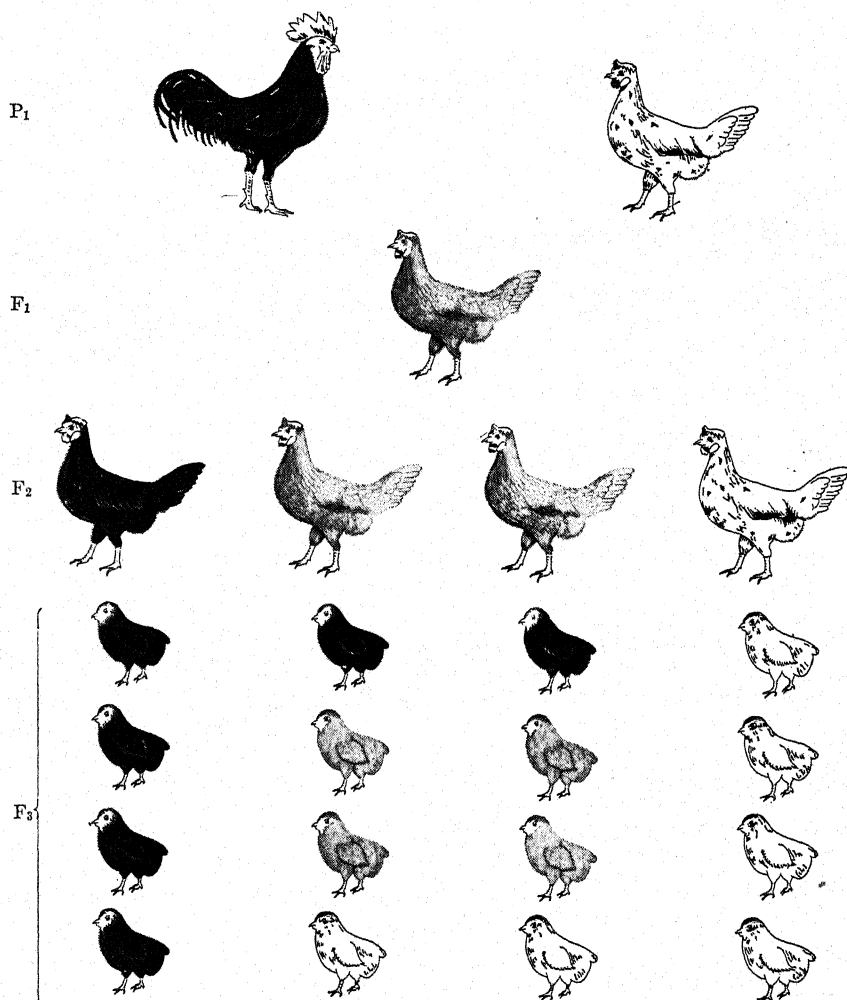


FIG. 17. The inheritance of plumage color in Andalusian fowls. Crosses of black by splashed-white produce only blue progeny in F₁; these, when bred together, produce in F₂, $\frac{1}{4}$ black which breed true, $\frac{1}{2}$ blue which breed like the F₁ blue, and $\frac{1}{4}$ white which breed true.

is incomplete. There may thus be all stages between complete dominance and the absence of dominance; and these various conditions may all be found among the different traits of a single individual. The rule which

holds regardless of whether dominance is or is not complete is, however, this: Provided that the parents belong to true breeding varieties, the first-generation (F_1) hybrids are all uniform in their hereditary traits.

Segregation. In contrast with the uniformity of the first-generation hybrids, the second generation, produced by self-fertilization of the F_1 red-flowered plants, consisted of two different kinds of plants: red ones like the red grandparent and white ones like the white grandparent. No other kinds of plants appeared, that is, none were found with dilute red flowers or pink or rose or other intermediate shades. In succeeding generations these same two types of flower color maintained their distinctness. In the F_2 generations bred from the hybrids between the other pairs of contrasting characters, similar results were obtained, that is, the traits of both parents of the original cross reappeared in different individuals (Table II). These pairs of contrasting characters Mendel called "differentiating characters." Such pairs of characters were later termed *allelic* pairs, each of the two members being the allele of the other. Thus, the red flower color in peas is allelic to white, round form of seed allelic to wrinkled, etc.

TABLE II. SUMMARY OF F_2 RESULTS OF MENDEL'S EXPERIMENTS WITH PEAS

Character	Dominants		Recessives		Total
	No.	%	No.	%	
Form of seed.....	5,474	(74.74)	1,850	(25.26)	7,324
Color of cotyledons.....	6,022	(75.06)	2,001	(24.94)	8,023
Color of seed coats, and flowers.....	705	(75.90)	224	(24.10)	929
Form of pod.....	882	(74.68)	299	(25.32)	1,181
Color of pod.....	428	(73.79)	152	(26.21)	580
Position of flowers.....	651	(75.87)	207	(24.13)	858
Length of stem.....	787	(73.96)	277	(26.04)	1,064
	14,949	(74.90)	5,010	(25.10)	19,959

When the F_1 hybrids are intermediate between the parents, as in the case between crosses between red and white snapdragons or between black and white Andalusian fowls (Figs. 16 and 17), the F_2 generation contains three types of individuals: those resembling both parental forms and those resembling the F_1 hybrids. No other intermediate color types appear. Just as the constant feature of F_1 hybrids is their uniformity, the individuals of the second (F_2) generation always differ in the same way as the parents did. This reappearance of the parental characters Mendel ascribed to the *segregation of the members of a pair of alternative or allelic characters* received from the parents. The genotypes of the parents are, consequently, not blended in the hybrids; instead, they preserve their individualities and *segregate* in the offspring.

Mendel fully realized the fundamental importance of this, for the occurrence of segregation proves that a phenotypic character of an organism, such as the red flower color or the round seed form, is produced by something which is transmitted from parents to offspring through the gametes and which ultimately determines the appearance of the character in the organism coming from the union of these gametes. This "something" is now called a *gene*. The allelic genes for contrasting characters, such as those for red and white flowers or round and wrinkled seeds, do not blend, contaminate, or absorb each other while they are together in the hybrid organism, and this is quite regardless of whether the phenotype of the hybrid shows complete or incomplete dominance or no dominance at all. Instead, the genes segregate, uncontaminated, and pass to different individuals in the offspring of a hybrid.

very fine work
The Mendelian Ratios. Mendel, unlike his predecessors, counted the numbers of individuals with each of the two differentiating characters which reappeared by segregation in F_2 , and this led him to the discovery of the explanation of the phenomenon of segregation. In the experiment with flower color, for example, he raised 929 F_2 plants and found that 705 of them bore red flowers and 224 bore white flowers. A similar segregation occurred in the F_2 generations from crosses involving other pairs of differentiating characters, and when all these counts were compared, the same simple ratio was found in each—that is, about $\frac{3}{4}$ of the F_2 resembled the dominant grandparent and $\frac{1}{4}$ resembled the recessive one (Table II). Later work on peas by other investigators has completely confirmed Mendel's results.

In the cross of red and white peas, let C stand for the gene for red flowers and c for the allelic gene for white flowers. Now, since an individual arises from the union of two gametes, it receives a gene for flower color from both parents. The true-breeding red-flowered parent may, therefore, be represented as CC and its gametes as C ; the true-breeding white parent is cc , and its gametes are c (Fig. 18). When the two plants are crossed, an egg, C , is fertilized by a male gamete, c (or vice versa), the resulting hybrid zygote will have both C and c and its "genetic formula" will be Cc . When the two members of a given pair of alleles carried in an individual are alike, this individual is said to be a *homozygote*. The true-breeding red (CC) and white (cc) plants are homozygotes. When the two members of an allelic pair are unlike, we are dealing with a *heterozygote*. The red-flowered plants obtained by Mendel in the F_1 generation (Cc) were heterozygotes. They were red because the gene C is dominant over c .

According to the principle of segregation, the two genes borne in the heterozygous Cc plants do not fuse or contaminate each other, despite the fact that the phenotype of this hybrid shows only the red flower color and fails to show any visible indication of the presence of the gene c in the geno-

type. Let us assume that these allelic genes segregate when the hybrid organism forms its gametes, so that approximately half of the gametes will carry C and the other half c . Let us assume further that in fertilization the gametes combine at random, so that there is no preference for or avoidance of unions of gametes which contain like or unlike genes. Finally, let it be supposed that the plants of all genotypes, CC , Cc , and cc are equally viable,

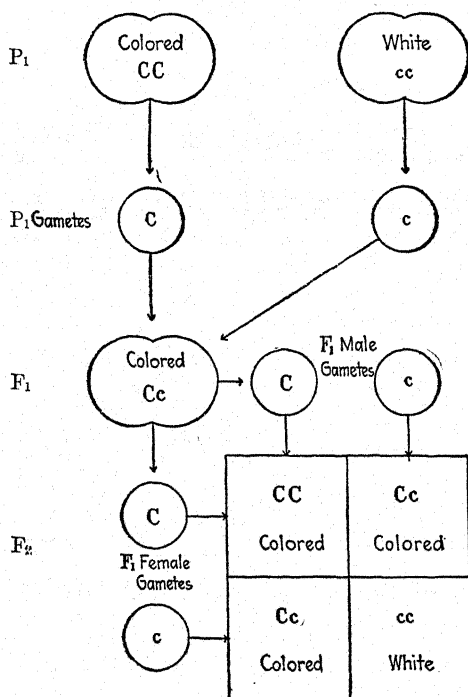


FIG. 18. Chart showing the behavior of the genes in the cross illustrated in Fig. 15 (colored and white flowers in peas), and giving the genotypes and phenotypes of parents and F_1 , the gametes which they produce, and the random union of F_1 gametes to form the three genotypic and two phenotypic classes of F_2 zygotes shown in the checkerboard.

so that the relative proportions of these plants grown from seeds are the same as the proportions formed at fertilization. As shown in Fig. 18, male gametes with C may fertilize C eggs, forming CC plants; C male gametes may fertilize c eggs, forming Cc plants; c male gametes with C eggs give also Cc plants; finally, c male gametes and c eggs produce cc plants. Since each of these four combinations is just as apt to occur as any of the other, each should give rise to approximately one-fourth of the progeny. The homozygous CC plants have only the gene for the red flower color and will

therefore have red flowers; Cc plants have a gene for red and one for white color, but since C is dominant over c , these plants will have red flowers also; cc plants have two genes for white color and will produce only white flowers. Hence, if the assumptions stated above are valid, about three-quarters of the plants in the F_2 generation should have red flowers and about one-quarter should have white flowers. This is the same as to say that the red-flowered and the white-flowered plants should appear in a ratio of approximately 3:1.

Among 929 plants actually obtained by Mendel in the F_2 generation of the red \times white cross, 705 had red and 224 had white flowers, thus displaying

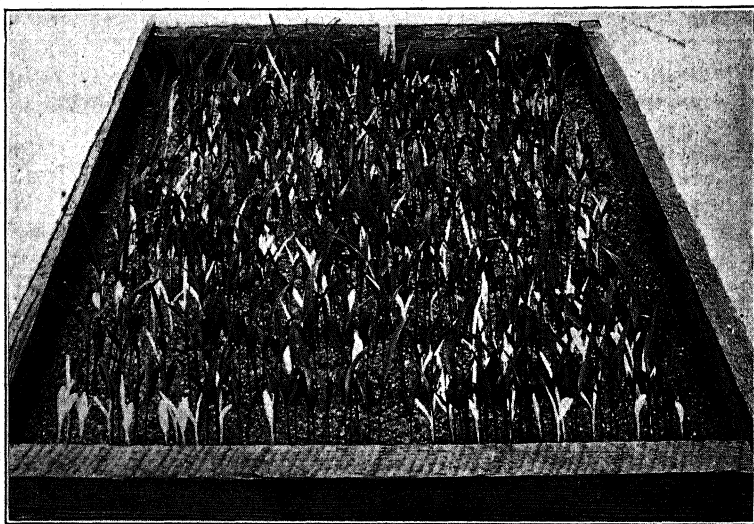


FIG. 19. The 3:1 ratio in an F_2 generation. Seedlings from a cross of two green plants each carrying a recessive albino gene, showing segregation into $\frac{3}{4}$ green and $\frac{1}{4}$ albino plants. (From Connor and Karper, in *Jour. Heredity*.)

a segregation of 75.90 per cent red and 24.10 per cent white-flowered plants (Fig. 18 and Table II). This is close to the expected ratio of $\frac{3}{4}:\frac{1}{4}$, or 3:1, or 75:25 per cent, whichever way we choose to express it. Very similar ratios were obtained by Mendel in crosses involving all the other characters studied. The actual counts which Mendel obtained in these various crosses are set forth in Table II. In the crosses involving yellow seeds and green seeds, F_2 generations totaling 179,399 seeds have been recorded by other investigators (Table III). Among these seeds 134,707, or 75.09 per cent, were yellow, and 44,692, or 24.91 per cent, were green. Other examples of this Mendelian ratio are shown in Figs. 19 and 20.

Of course, the results of breeding experiments do not display exact ratios

any more than the tossing of coins or throwing of dice always gives exactly the same results; and the reason in each case is the same. It must not be expected, for example, that with every three red-flowered plants there shall always be associated one with white flowers, any more than that in tossing coins heads will invariably alternate with tails. The ratio 3:1 and other Mendelian ratios merely indicate the expectation on the basis of probability. And indeed, the larger the number of individuals raised, the closer the actually observed ratios tend to approach to the ideal ones (Table III).

TABLE III. SUMMARY OF F_2 RESULTS IN INHERITANCE OF SEED COLOR IN PEAS
(After Johannsen)

Investigator	Yellow seeds		Green seeds		Total
	No.	%	No.	%	
Mendel, 1865.....	6,022	(75.05)	2,001	(24.95)	8,023
Correns, 1900.....	1,394	(75.47)	453	(24.53)	1,847
Tschermak, 1900.....	3,580	(75.05)	1,190	(24.95)	4,770
Hurst, 1904.....	1,310	(74.64)	445	(25.36)	1,755
Bateson, 1905.....	11,902	(75.30)	3,903	(24.70)	15,806
Lock, 1905.....	1,438	(73.67)	514	(26.33)	1,952
Darbishire, 1909.....	109,060	(75.09)	36,186	(24.91)	145,246
Totals.....	134,707	(75.09)	44,692	(24.91)	179,399

Phenotypic and Genotypic Ratios. Mendel was not content to leave the matter here but applied further and very rigorous tests to his conclusions and interpretations. In the F_2 of his cross of red-flowered to white-flowered peas, there were approximately 75 per cent of plants with red and 25 per cent with white flowers. The red and white phenotypes appeared in a ratio approaching 3:1. But Mendel's interpretation of these results, illustrated in the chart in Fig. 18, suggests that there must be two kinds among the red-flowered F_2 plants. About one-third of them should be genotypically CC , that is, homozygous for the gene for the red flower color, and about two-thirds should be heterozygotes, Cc , carrying both the dominant gene C and the recessive c . The white-flowered plants should all be recessive homozygotes, cc . The validity of these predictions can be tested by experiments. The homozygous white-flowered plants should all breed true to white flower color through all subsequent generations if self-fertilized or crossed to each other. The red-flowered plants, however, although looking alike, should not all behave in the same way. About one-third of them, namely, those homozygous for the gene C (hence, having the genotypic formula CC , see Fig. 18) should breed true to red. But two-thirds of

the reds, namely, the heterozygotes, Cc , should breed exactly like the F_1 hybrid plants, that is, they should produce red and white offspring in the ratio of about $\frac{3}{4}$ red: $\frac{1}{4}$ white. This is, indeed, what happened in the experiments (Fig. 15).

The phenotypic segregation ratio in the F_2 of the cross of red to white is, therefore, 3 red:1 white, but the genotypic segregation ratio is 1 homo-

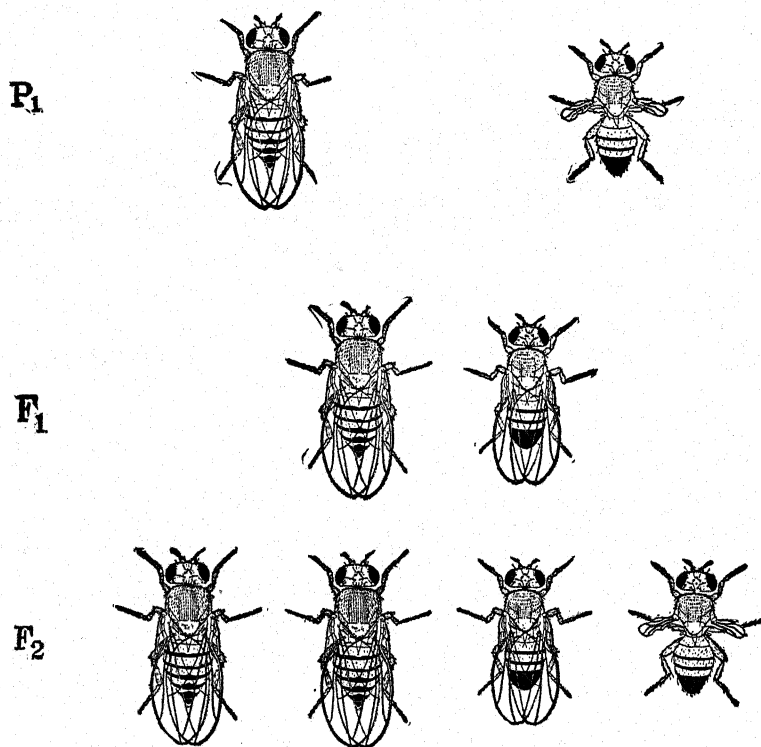


FIG. 20. Cross between long-winged (wild-type) vinegar fly and vestigial-winged fly, producing long-winged offspring in the F_1 , which if bred to each other give in the next generation (F_2) $\frac{3}{4}$ long to $\frac{1}{4}$ vestigial. (From Morgan.)

zygous red:2 heterozygous reds:1 homozygous white (or $\frac{1}{4} CC:\frac{1}{2} Cc:\frac{1}{4} cc$: or 25:50:25 per cent). The difference between the visible and actual ratios is due to dominance of C over c , which causes the same phenotype to develop from the CC and Cc genotypes.

However, dominance is not observed in some crosses, such as those of red and white snapdragons (Fig. 16) and of black and splashed-white Andalusian fowls (Fig. 17). In these crosses, the heterozygotes are phenotypically distinguishable from both homozygotes. Accordingly, the pheno-

typic segregation ratios in the F_2 of these crosses must reflect the genotypic ratios. Ratios of 1 red:2 pink:1 white in snapdragons and of 1 black:2 blue:1 splashed white in Andalusian fowls are expected. These ratios have actually been observed.

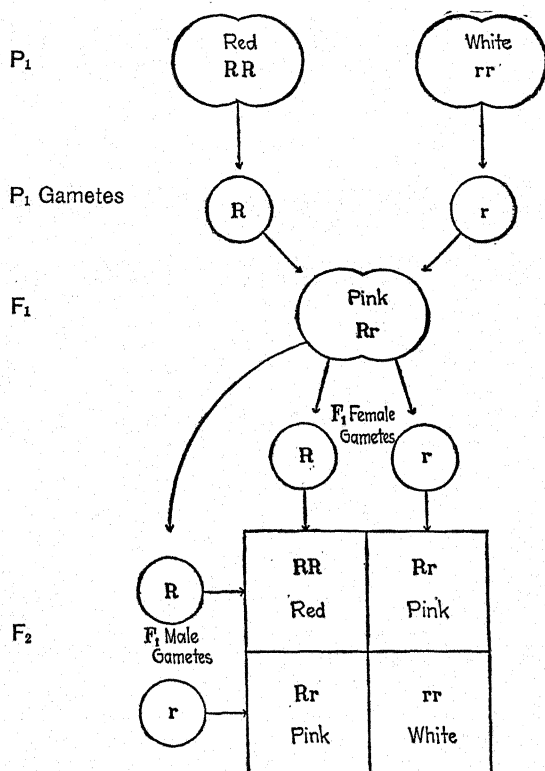


FIG. 21. Chart showing the behavior of the genes in the cross illustrated in Fig. 16 (red and white flowers in snapdragons) giving the genotypes and phenotypes of parents and F_1 , the gametes which they produce, and the random union of F_1 gametes to form the three genotypic and three phenotypic classes of F_2 zygotes shown in the checkerboard.

Test-cross Ratio. The validity of any scientific theory is best shown by experimental verification of predictions based on that theory. Mendel's theory is indeed able to serve as a basis for such verifiable predictions. Among such predictions made and verified by Mendel himself was the behavior of so-called *test crosses*, or *backcrosses*. The red-flowered F_1 hybrid plant from the cross of red \times white peas was *crossed back* to one of the parental types, namely, the recessive white-flowered one. The F_1 hybrid is Cc , and the white-flowered parent is cc . Now the gametes produced by

a hybrid are always *pure*, in the sense that each may carry either the allele C or c , but never a hybrid genotype. Furthermore, half of the gametes of a hybrid red-flowered plant should carry C , and the other half should have c . The gametes of a homozygous recessive white-flowered plant are, of course, all c . Thus, if pollen from the hybrid is placed on the stigma of a white-flowered plant, or vice versa, there is one chance in two that a C gamete will fertilize a c one and one chance in two that two c gametes will unite. The expected progeny would thus be heterozygous red-flowered (Cc) and white-flowered plants (cc) in equal numbers. This proved to be actually the case.

Thousands of such test crosses have produced 1:1 ratios in the offspring. When a heterozygote is phenotypically different from both homozygous parents, two complementary test crosses can be made. For example, if a pink-flowered snapdragon (Fig. 21) is backcrossed to the red parent, the progeny segregates in a ratio close to 1 red:1 pink. The cross pink \times white results in a segregation of 1 pink: 1 white.

Time When Segregation Takes Place. The law of segregation, otherwise known as *Mendel's first law*, states that allelic genes in a zygote do not blend or contaminate each other but segregate and pass into different gametes. The precise time when segregation takes place has been determined by inference from studies on the behavior of chromosomes at meiosis (see Chap. VII) and is now known to occur at the meiotic divisions. However, in some organisms it is possible to prove by direct observation that the segregation has taken place before a certain stage of the life cycle has been reached.

In maize, rice, peas, and some other plant species, there exist varieties which differ from each other in the kind of carbohydrate reserve materials which accumulate in the cells. For example, maize varieties known as "starchy" have starch grains which stain intense blue with iodine solutions, whereas the starch grains of "waxy" stain red. The difference is due to a single pair of allelic genes, the gene for starchy being dominant over waxy. The F_1 generation from a cross of starchy by waxy consists of starchy plants, and in F_2 a segregation of 3 starchy:1 waxy is observed. Carbohydrate reserves are laid down in many cells, including those of the pollen grains. Therefore, pollen grains of starchy maize stain blue and of waxy, red with iodine. The noteworthy fact is that the pollen grains of F_1 hybrid plants, which must be heterozygous for starchy and waxy, fall clearly into two about equally numerous classes, some staining blue and others red (Fig. 23). Among the starchy plants obtained in F_2 , about one-third should, theoretically, be homozygous for starchy, and two-thirds should be starchy-waxy heterozygous. This prediction is fully confirmed by finding that the pollen grains of about a third of the plants stain uniformly blue,

while about two-thirds of the plants give blue and red pollen grains in about equal numbers.

Still clearer is Mendelian segregation in spores of certain fungi. In some species of the pink bread mold (*Neurospora*) numerous mutations were

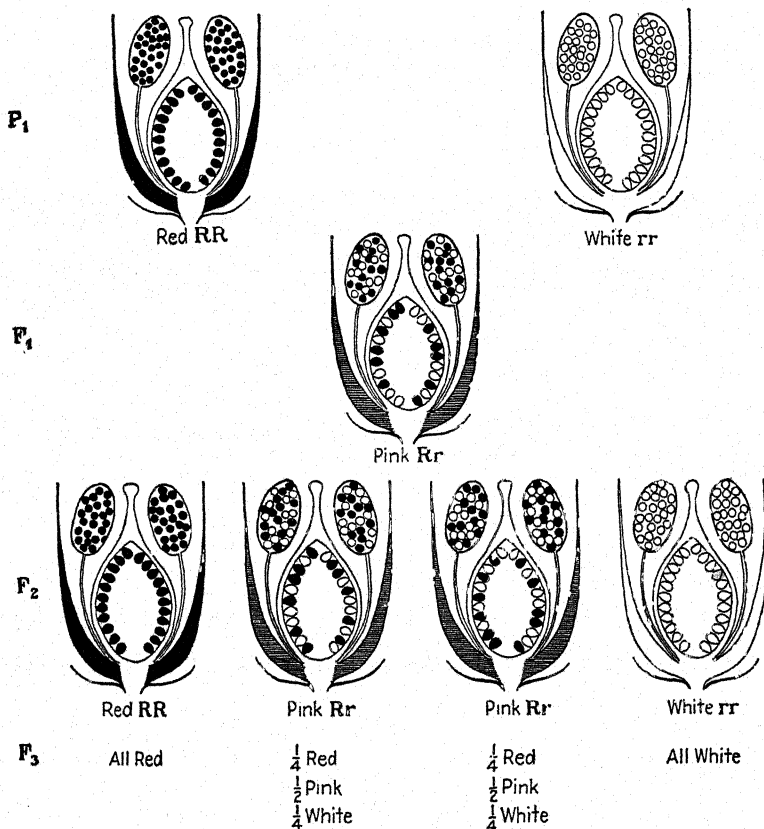


FIG. 22. Diagram showing the character of the gametes in three generations following a cross between a red-flowered and white-flowered plant. The gametes are represented by pollen grains and ovules, the black ones carrying the factor for red and the white ones that for white. In the (pink) F₁ dominance of red is not complete (corolla shaded), but of the F₁ gametes half carry red and half white, and none are pink. In the F₂ one-fourth of the plants are red-flowered, and all their gametes carry the gene for red; one-half are pink-flowered, with half their gametes carrying red and half white; and one-fourth white-flowered, their gametes all carrying white. The character of the offspring of these F₂ types, when self-fertilized, is shown in the F₃ generation.

observed, which gave rise to varieties differing in such traits as color of the mycelia, manner of growth, and especially in nutritional requirements for certain substances such as vitamins. The varieties can be crossed, because if two mycelia of opposite "sex" are brought in contact they establish

fusions in which the cell nuclei derived from the two individuals unite to form zygotic nuclei. The cells with zygotic nuclei then develop into so-called fruiting bodies (perithecia), which contain more or less numerous sacs, or *asci*, each ascus with eight spores (Fig. 124, p. 272). It is known that each spore, like the gametes, has only half of the chromosomes which were present in a zygotic nucleus. The eight spores of an ascus can be isolated with the aid of delicate needles under a microscope, and mycelia from each spore can then be grown in separate cultures. If the individuals crossed differed in a single trait, for example if one has a pink and the other a white

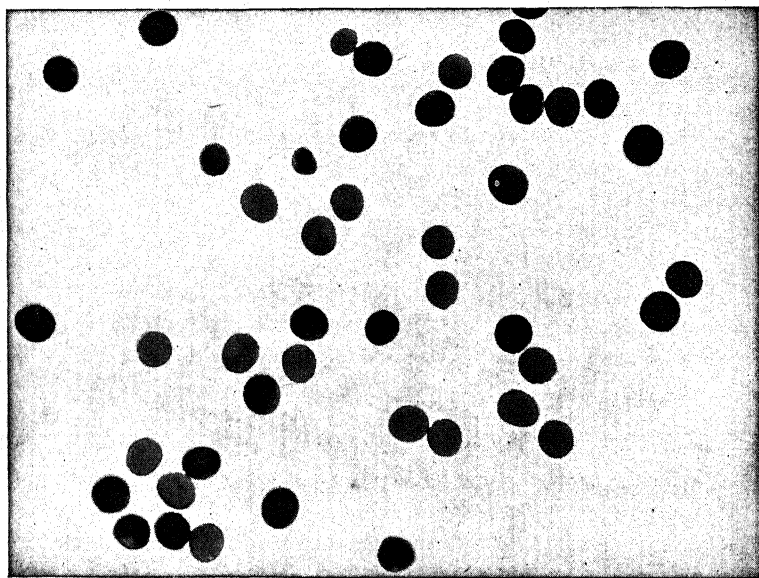


FIG. 23. Microphotograph of pollen from an anther of a maize plant heterozygous for the starchy-waxy gene pair showing segregation for this gene. The pollen has been treated with iodine making the "starchy" grains appear dark in contrast to the "waxy" ones, which are light. (From Demerec.)

mycelium, four of the eight spores of an ascus give pink mycelia and the other four give white mycelia. The segregation ratio is, hence, 1 pink:1 white. It is noteworthy that in this case the segregation is always such that four spores of an ascus carry one allele and the other four the alternative allele. This is because the eight spores of an ascus are the products of a single act of segregation (meiosis).

Measuring the "Goodness of Fit" of a Ratio. Obviously, the segregation ratios observed in breeding experiments will only rarely be exactly the ones that are theoretically expected, and it is important to know whether a given deviation from expectation is due merely to chance or whether some

other factor is operating which has caused a really significant departure. To determine this it is useful to calculate *the probable frequency with which a deviation as great as, or greater than, the observed one will appear if another similar trial is made or another similar sample taken.* The higher this frequency is, of course, the more likely is it that the deviation is due to chance alone, and the more likely is it that the population is really an instance of the particular ratio chosen.

This probability may be found by comparing the size of the deviation from the expected ratio with the size of what is known as the *standard error* of this ratio (S.E._r). The standard error of a ratio may be calculated from the following formula:

$$\text{S.E.}_r = \sqrt{\frac{pq}{n}}$$

where p is one of the theoretical percentages, q the other (necessarily equaling $1 - p$), and n the total number of individuals. We shall discuss the standard error and its significance in Chapter VI.

Let it be assumed, for example, that in a given F_2 population, where segregation in a ratio of $\frac{3}{4}:\frac{1}{4}$ is expected, there are actually found 390 individuals showing the dominant trait and 110 the recessive. This is a ratio of .78 to .22, and deviates from the expected ratio of .75 to .25 by .03. The standard error for this theoretical proportion will thus be

$$\sqrt{\frac{.75 \cdot .25}{500}} \text{ or } .0194$$

The deviation is therefore 1.55 times as great as its standard error ($.03/.0194 = 1.55$).

The deviation may also be compared with its standard error in terms of actual numbers by use of the formula

$$\text{S.E.}_r = \sqrt{\frac{C_1 C_2}{n}}$$

where C_1 is the expected number in one class and C_2 in the other. In the above example these are 375 and 125, so that

$$\text{S.E.}_r = \sqrt{\frac{375 \cdot 125}{500}} = 9.68$$

The actual deviation is, of course, $15(390 - 375)$; as before, it is 1.55 times its standard error ($15/9.68 = 1.55$).

How frequently a deviation of this relative size may be expected as a result of chance alone can be calculated from Table IV. The first column of the table gives the relative size of the deviation in terms of its standard error and is merely the deviation divided by its error. The second column shows the calculated percentage frequency with which a deviation of this

size, or larger, may be expected to occur again under similar circumstances as a result of chance alone. Thus a deviation of 1.6 or more times its error may be expected in 10.96 per cent of similar cases; that is, it has a probability of about 11 per cent, or .11. A deviation of 1.55 times its error (the example given above) will occur somewhat more often than this, actually in about 12 per cent of the cases. Such a relationship may also be expressed as 1 chance out of 8, or the odds against its occurrence may be given as 7:1.

TABLE IV

Deviation S.E.	Probability of occurrence per 100 trials*	<i>p</i>	Odds against its occurrence (approximate)
1.0	31.74	.31	2.1:1
1.1	27.14	.27	2.7:1
1.2	23.02	.23	3.3:1
1.3	19.37	.19	4.2:1
1.4	16.16	.16	5.2:1
1.5	13.37	.13	6.5:1
1.6	10.96	.11	8.1:1
1.7	8.91	.09	10.2:1
1.8	7.18	.07	12.9:1
1.9	5.74	.05	16.4:1
2.0	4.56	.04	21:1
2.1	3.57	.04	27:1
2.2	2.78	.03	35:1
2.3	2.14	.02	46:1
2.4	1.64	.02	60:1
2.5	1.24	.01	80:1
2.6	.94	.009	106:1
2.7	.69	.007	143:1
2.8	.52	.005	195:1
2.9	.37	.003	267:1
3.0	.26	.003	369:1
4.000006	16,000:1

* Actually the figures in this column represent the percentage of the total area of the normal curve lying *outside* the limits of the mean plus the given multiple of the standard deviation, or S.E. deviation/S.E., and the mean minus this multiple. This value defines the likelihood that a deviation as great as or greater than the one specified on each line of the table will be found in 100 trials having the same significance as *p* in Table VIII. (Table of χ^2 & *p*).

In general, if the deviation of a ratio from the expectation is so great that it is more than twice the standard error of this ratio, it is commonly regarded as significant, since in only about 4.5 per cent of similar cases will as great

a deviation occur by chance. Thus in crossing solid-colored rats known to be heterozygous for a recessive gene for a white spotting pattern known as hooded, with the recessive parent type, $Hh \times hh$, Roberts, Dawson, and Madden obtained 552 solid-colored and 448 hooded offspring. This is a deviation of 52 from the expected number of 500 in each class. The standard error is $\sqrt{(500 \cdot 500)/1,000}$ or $\sqrt{250} = 15.81$. The ratio, deviation/S.E., is $52/15.81$, or 3.3. A deviation of this size has a probability of occurrence, due to chance alone, of less than .0026; that is to say, there is less than a .26 per cent chance that the deviation is accidental. Another way of saying this is that the chance of occurrence of such a deviation is about 1 in 360, so that the odds against it are about 360:1. The actual ratio apparently does not "fit." This probably means that one of the assumptions on which the 1:1 expectation was based is wrong in this particular case. Since it was known from other evidence that the genes H and h do show Mendelian segregation and that, in all experiments involving this pair, too few hooded (hh) animals are found, it is likely that the homozygous recessive genotype hh is less viable than H , that is, that more hooded than nonhooded rats die before birth.

A segregating population that deviates from a given expected ratio by less than twice its standard error is considered an instance of the ratio in question. The deviation in the first example cited above is thus probably not significant since one as great would be expected 12 per cent of the time. The population is therefore probably segregating in the ratio of 75:25 per cent, as was expected. The size of a deviation from expectation in proportion to its error may thus be used as a measure of the "goodness of fit" of any observed ratio to any given theoretical expectation. This general subject is discussed again in Chapters III and VI.

Segregation in Populations. Up to this point we have considered the phenomena of Mendelian segregation as they are observed in the offspring of experimental crosses. The same law is operating in populations which contain different proportions of individuals homozygous and heterozygous for various genes, such as human populations or the wild and cultivated species of animals and plants. This can be readily proved by comparing the ratios of both types of homozygotes and of heterozygotes which are found in populations in which matings or marriages occur at random with respect to a particular gene, with the ratios expected to result from segregation and random combination. In some populations not amenable to experimental breeding, for example, human populations, the principle of segregation can be deduced from the ratios of homozygotes to heterozygotes. This important corollary of Mendel's first law, which has led to the "gene-frequency method" of studying heredity, is discussed in detail in Chapter XII. A preliminary understanding of it can be gained by solving Problems 51 and 52 at the end of this chapter.

Segregation in Inbred Populations. One important conclusion about genes in certain kinds of populations was derived by Mendel himself, likewise as a corollary of the principle of segregation. He considered the case which would arise in the normally self-fertilized plants with which he worked, in which both uniting gametes came from the same parent. A simple calculation showed that a population consisting of Cc plants would, under self-fertilization, quickly come to consist chiefly of CC and cc plants according to a regular law of decrease of heterozygotes. This law, which was formulated in Mendel's first paper, forms the basis for the present theory of the effect of inbreeding, which is discussed in Chapter XII.

Segregation in Pedigrees. In cases in which it is impossible to test the occurrence of segregation by experiment or by the gene-frequency method, it is sometimes possible to recognize and prove the segregation of a gene by following its effects in the several generations of a family tree or pedigree. This has to be resorted to most often in human families, and until recently

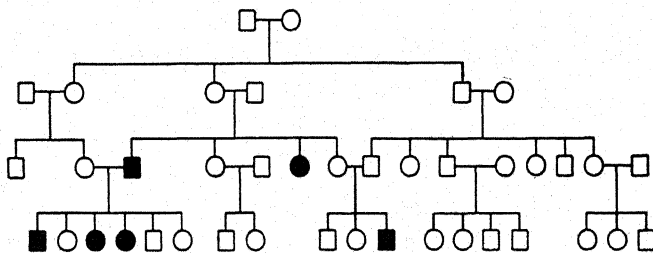


FIG. 24. A pedigree of albinism. Squares represent males, circles females. Open symbols—persons not showing the trait in question; black symbols—persons showing the trait (albinos).

most of our knowledge of heredity in man came from pedigree studies. Characters to be studied in this way must be subject to easy division into sharply alternative classes, since in a pedigree in which segregation is to be tested we can designate only two conditions represented by AA and Aa as one type and aa as the other, where A is dominant, or as three distinct types, where Aa is intermediate. The sample pedigree of albinism (Fig. 24) illustrates such a character. It is obvious that albinism is recessive since only two phenotypes occur and albino children are born from marriages of normal parents, but never the reverse. The parents of the albinos are also related as first cousins, and wherever a recessive gene is rare, as that for albinism is, it is to be expected that two Aa persons who become the parents of an aa (albino) will have received the rare gene from the same source, that is, from a common ancestor. From this pedigree it is possible to calculate the chance that any individual will transmit an albino gene and, thus, the chance that an albino child will be born from a specified marriage within the family (see legend, Fig. 24).

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PROBLEMS

23. What advantage has the method of experiment over that of observation alone as a means of studying natural phenomena?
24. What advantage and what disadvantage have plants over animals as material for the study of heredity?
25. Explain how it can be that individuals which look very much alike breed very differently. — phenotyp
- ✓ 26. Why is the F_1 between two homozygous parents as uniform as the parents themselves?
27. In human families traits are often observed to "skip" a generation or two. How do you explain this?
- ✓ 28. Which do you think would be easier to handle in breeding, a trait which shows complete dominance or one which does not? Why?
29. What evidence is there that genes occur in the body cells as well as in the gametes?
30. Does Mendelian segregation take place in asexual reproduction? Explain.

31. Why is the principle of Segregation of more fundamental importance than the principle of Dominance? ✓

✓ 32. In man why are parents of rare recessives likely to be related?

33. Does segregation occur in homozygotes?

Note. In summer squashes white fruit color is dominant over yellow.

34. If a squash plant homozygous for white is crossed with one homozygous for yellow, what will be the appearance of the F_1 ? of the F_2 ? of the offspring of a cross of the F_1 back to its white parent? of the offspring of a cross of the F_1 back to its yellow parent?

35. Let the allele for white fruit be represented by W and that for yellow by w . What kind of gametes as to fruit color will be produced by plants of the genotypes WW , Ww , and ww ? ✓

36. What gametes will be produced by the plants involved in the following crosses, in which the genotypes of the parents are given, and what will be the fruit color of the offspring from each cross: $Ww \times ww$; $WW \times Ww$; $ww \times WW$; $Ww \times Ww$? ✓

37. A white-fruited squash plant when crossed with a yellow-fruited one produces offspring about half of which are white and half yellow. What are the genotypes of the parents? ✓

38. If the white-fruited parent in the preceding question is self-fertilized, what will be the fruit color of its offspring? ✓

39. Two white-fruited squash plants when crossed produce about three-fourths white and one-fourth yellow offspring. What are the genotypes of these two parents? What will each produce if crossed with a yellow-fruited plant? ✓

40. A cross between a white-fruited and a yellow-fruited squash plant produces all white plants. If two of these F_1 white plants are crossed together, what will be the appearance of *their* offspring? ✓

Note. The polled or hornless condition in cattle, P , is dominant over the horned, p .

41. A certain polled bull is bred to three cows. With cow A, which is horned, a polled calf is produced; with cow B, also horned, a horned calf is produced; with cow C, which is polled, a horned calf is produced. What are the genotypes of these four animals, and what further offspring would you expect from these three matings?

Note. In man, brown eyes, B , are dominant over blue, b .

42. A brown-eyed man marries a blue-eyed woman and they have eight children, all brown-eyed. What are the genotypes of all the individuals in the family?

43. A blue-eyed man both of whose parents were brown-eyed marries a brown-eyed woman whose father was brown-eyed and whose mother was blue-eyed. They have one child, who is blue-eyed. What are the genotypes of all the individuals mentioned?

44. What are the chances that the first child of a marriage of two heterozygous brown-eyed parents will be blue-eyed? If the first child is brown-eyed, what are the chances that the second child will be blue-eyed?

Note. In four-o'clock flowers, red flower color, R , is incompletely dominant over white r , the heterozygous plants being pink-flowered.

45. In the following crosses, in which the genotypes of the parents are given, what are the gametes produced by each parent, and what will be the flower color of the offspring from each cross: $Rr \times RR$; $rr \times Rr$; $RR \times rr$; $Rr \times Rr$?

46. If a red-flowered four-o'clock plant is crossed with a white-flowered one, what will be the flower color of the F_1 ? of the F_2 ? of the offspring of a cross of the F_1 with its red parent? with its white parent?

47. If you wanted to produce four-o'clock seed *all* of which would yield pink-flowered plants when sown, how would you do it?

Note. In Andalusian fowls the heterozygous condition of the alleles for black plumage (B) and white (b) is blue.

48. What offspring will a blue Andalusian fowl have if bred to birds of the following plumage colors: (1) black; (2) blue; (3) white?

Note. In poultry, rose comb is dominant over single comb.

49. A farmer believes that some of his rose-combed Wyandotte fowls may carry a factor for single comb. Can you suggest a method for finding out which fowls are heterozygous?

50. Two black female mice are crossed with a brown male. In several litters female 1 produced 9 blacks and 7 browns; female 2 produced 57 blacks. What deductions can you make concerning inheritance of black and brown coat color in mice? What are the genotypes of the parents in this case?

51. Assume that in a particular species of plants colored flowers are dominant over white ones and that (as in beans) the flowers are *self-fertilized* in nature. Assume that one heterozygous colored-flowered plant, Cc , becomes established on an island where no other individuals of this species exist and that its offspring thrive and multiply there *in great numbers*. Assume also that it is an annual plant and that thus there is no chance for members of one generation to cross with those of another. What will the *fifth* generation of descendants look like as to flower color?

52. Make just the same assumptions as in Problem 51, *except* that the plant in question (like sunflowers and many other plants and animals) is *self-sterile* and must be crossed with another plant to set fertile seed; that two heterozygous plants, Cc and Cc , are the original invaders; and that the individuals of each generation breed freely together. What will the fifth generation of *these* plants look like as to flower color?

53. A given F_2 population consists of 404 A and 129 a individuals. Calculate the deviation of this segregation from a 3:1 ratio, the standard error of this ratio, and the ratio $d/S.E.$. Is this deviation significant? Does a single-factor segregation satisfactorily explain the result?

	A	a
$Aa \times Aa$	870	330
	40	20
	306	94

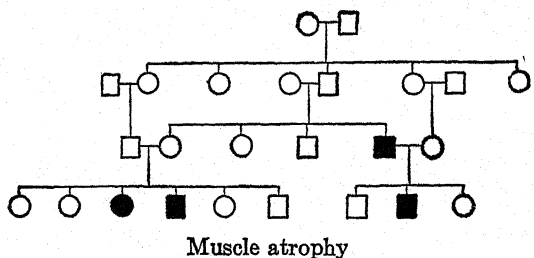
Note. In the following six human pedigrees the individuals which are solid black possess the trait mentioned. Squares represent males and circles females. Determine for each pedigree the *method of inheritance* of the trait in question (whether dominant or recessive); and, as far as possible, determine for that trait the *genotype of each individual* in the pedigree.

Left-handedness

Pedigree chart illustrating the inheritance of polydactyly across three generations (I, II, and III). The chart shows the transmission of the trait, with affected individuals (black symbols) and unaffected individuals (white symbols). The trait is labeled as Polydactyly.

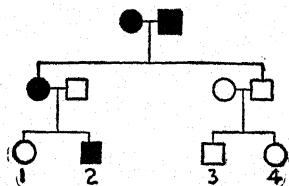
Monilothrix

59.



Note. In the following four pedigrees, calculate the probability that the trait in question will appear in the offspring of the various matings indicated. Assume that these individuals have had no children and that the only indication as to their genotype is the occurrence of the trait in the pedigree. Assume further (unless there is evidence to the contrary) that individuals who have married into these families and who do not show the trait in question do not carry recessive genes for it.

60.

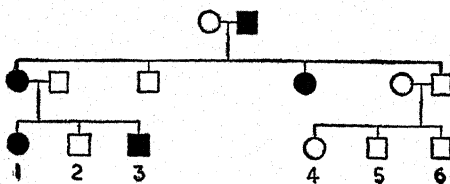


Trait dominant

1×3

2×4

61.

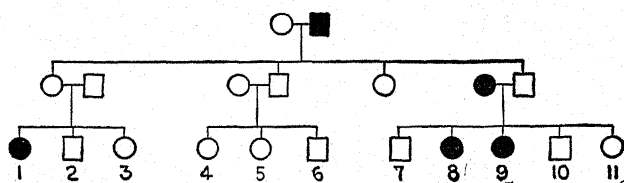


Trait dominant

1×5

2×4

62.



Trait recessive

1 × 6

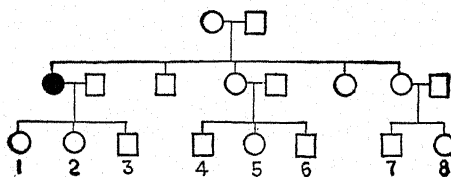
2 × 4

1 × 7

3 × 10

6 × 11

63.



Trait recessive

1 × 7

2 × 4

6 × 8

CHAPTER III

MENDEL'S PRINCIPLE OF INDEPENDENT ASSORTMENT

We have just seen how Mendel's wisdom in studying the inheritance of single characters separately led him to the discovery of the principle of segregation as discussed in the preceding chapter. This principle is concerned with the hereditary behavior of the members of a pair of alternative or allelic genes. The genotype of the individual, however, consists of many pairs of genes, and so it is necessary to be able to follow, not only a single character, but a whole series of them at once and to understand how they behave with relation to each other in their passage from generation to generation.

The Principle of Independent Assortment. Mendel studied seven pairs of characters in peas, involving seed color, seed surface, flower color, vine height, color of unripe pods, pod shape, and position of flowers. A study of the results of experiments in which plants differing in two or more of these characters were crossed led to his discovery of the second major principle of Mendelian inheritance, namely, that the different characters in the offspring of hybrids are distributed independently of each other.

Mendel was led to a recognition of this principle by the results of a cross made between a pea plant having round and yellow seeds with one having wrinkled and green ones.¹ In this case he found, of course, that the F_1 hybrids were all round-seeded and yellow-seeded, since these two characters had both proved to be dominant. When two of these F_1 hybrids were crossed, however (or when one of them was self-fertilized), and an F_2 generation raised therefrom, he found that in this generation there appeared not only the two original combinations of characters—round with yellow and wrinkled with green—but two *new* combinations, *round with green and wrinkled with yellow*. These four kinds of plants, moreover, were not equal in numbers but appeared in a definite ratio, the successful interpretation of which was Mendel's second great contribution to genetic theory. He raised 556 second-generation plants, and the counts which he obtained were as follows:

315 round and yellow.
108 round and green.

101 wrinkled and yellow.
32 wrinkled and green.

¹ Such a cross as this, which involves *two* character differences, is technically known as a *dihybrid* cross.

Considering either of these character pairs *alone*, it is found that approximately three-fourths of the plants show the dominant trait and one-fourth show the recessive, as a knowledge of the principle of segregation would

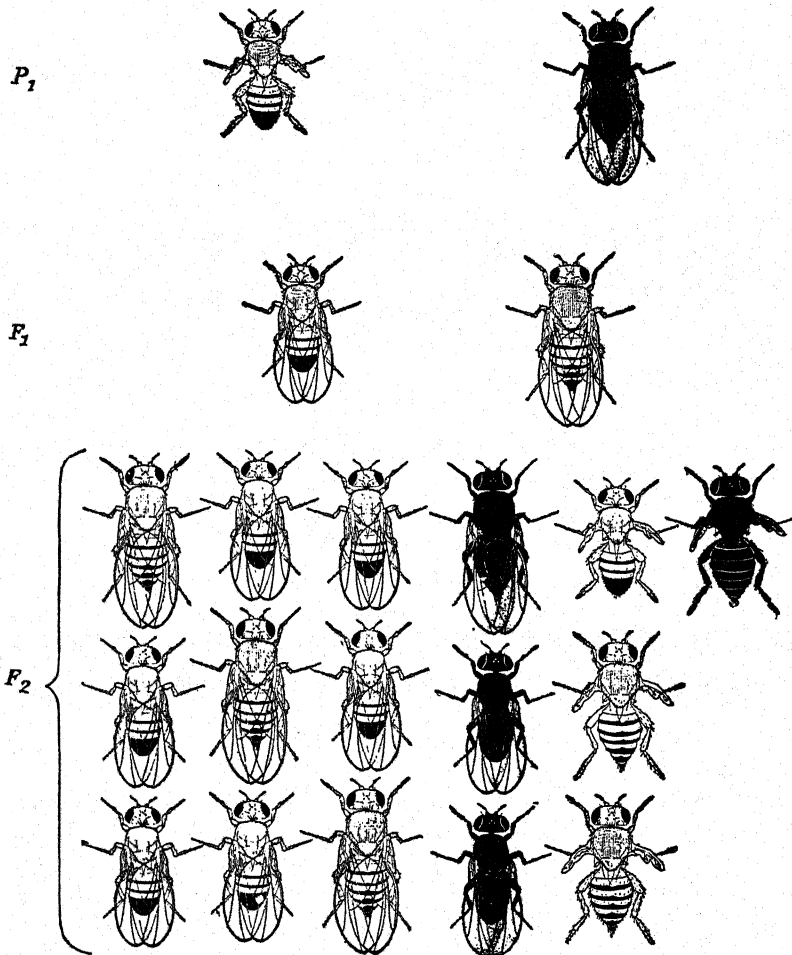


FIG. 25. The independent inheritance of two pairs of characters in *Drosophila*. A pure long-winged fly with ebony body mated with a vestigial-winged, gray-bodied one produces all long-winged, gray-bodied flies in F_1 . These when inbred produce an F_2 generation consisting of $\frac{9}{16}$ long, gray; $\frac{3}{16}$ long, ebony; $\frac{3}{16}$ vestigial, gray; and $\frac{1}{16}$ vestigial, ebony. (From Morgan, Sturtevant, Muller, and Bridges, courtesy of Henry Holt & Company.)

lead one to expect. Thus, of the total 556 plants 423, or 76.08 per cent, are round-seeded; 133, or 23.92 per cent, are wrinkled-seeded; 416, or 74.82 per cent, are yellow-seeded; and 140, or 25.18 per cent, are green-seeded.

When both character pairs are considered *together*, however, it is found that the segregation into three-fourths and one-fourth which occurs in each pair when considered alone is *entirely independent* of the similar segregation which takes place in the other pair. Thus, of the three-fourths of the entire group of plants which are round-seeded, approximately three-fourths, in turn, are yellow-seeded, and one-fourth are green; and of the other fraction (one-fourth) which are wrinkled-seeded, three-fourths, again, are yellow-seeded and one-fourth green. This leads to the result that *three-fourths of three-fourths* of the entire number of plants in the F_2 generation, or nine-sixteenths of the entire number of plants, show both dominant characters (round and yellow); *one-fourth of three-fourths*, or three-sixteenths, show one dominant and one recessive (round and green); *three-fourths of one-fourth*, or again three-sixteenths, show the other combinations of dominant and recessive (wrinkled and yellow); and only *one-fourth of one-fourth*, or one-sixteenth, show both recessive characters (wrinkled and green). The counts which Mendel actually obtained in his experiment (315:108:101:32) came very close to these proportions,¹ and he therefore inferred that the second generation from a cross involving two character pairs shows four kinds of individuals, approximately in the ratio of $\frac{9}{16}:\frac{3}{16}:\frac{3}{16}:\frac{1}{16}$, or 9:3:3:1. His results with other characters in peas and similar crosses, which have been made many times by others with various animals and plants, leave no doubt that this ratio is the true one for such dihybrid crosses involving independent characters which show dominance. Similar cases of dihybrid inheritance in *Drosophila* and squashes are shown in Figs. 25 and 26.

This *independent assortment* of two character pairs is made still more manifest by the fact that the particular combination in which the characters are brought into a cross makes no difference at all in the manner in which they are assorted and recombined in the F_2 . In the example cited both dominant characters were brought in by one parent and both recessives by the other, but exactly the same results are obtained in the F_2 if, instead of crossing round and yellow with wrinkled and green, round and green is crossed with wrinkled and yellow. The F_1 is round, yellow; and the F_2 is again $\frac{9}{16}$ round, yellow; $\frac{3}{16}$ round, green; $\frac{3}{16}$ wrinkled, yellow; and $\frac{1}{16}$ wrinkled, green.

Explanation of Independent Assortment. Perhaps it will be easier to understand what is involved in the principle of independent assortment if the genes are again represented by letters and the genotypes of the various individuals and the gametes which they form are studied in this way: Let the gene for round seeds be represented by *R* and its allele for wrinkled

¹ The actual may be compared with the perfect ratio by multiplying the total number of plants, in this case 556, by $\frac{9}{16}$, $\frac{3}{16}$, $\frac{3}{16}$, and $\frac{1}{16}$. The perfect ratio in this case is 312.75:104.25:104.25:34.75.

seeds by r ; and the gene for yellow seeds by Y and for green seeds by y . Mendel's original round, yellow parent plant would thus be represented by the formula $RR YY$, and his wrinkled, green plant by $rr yy$. It has

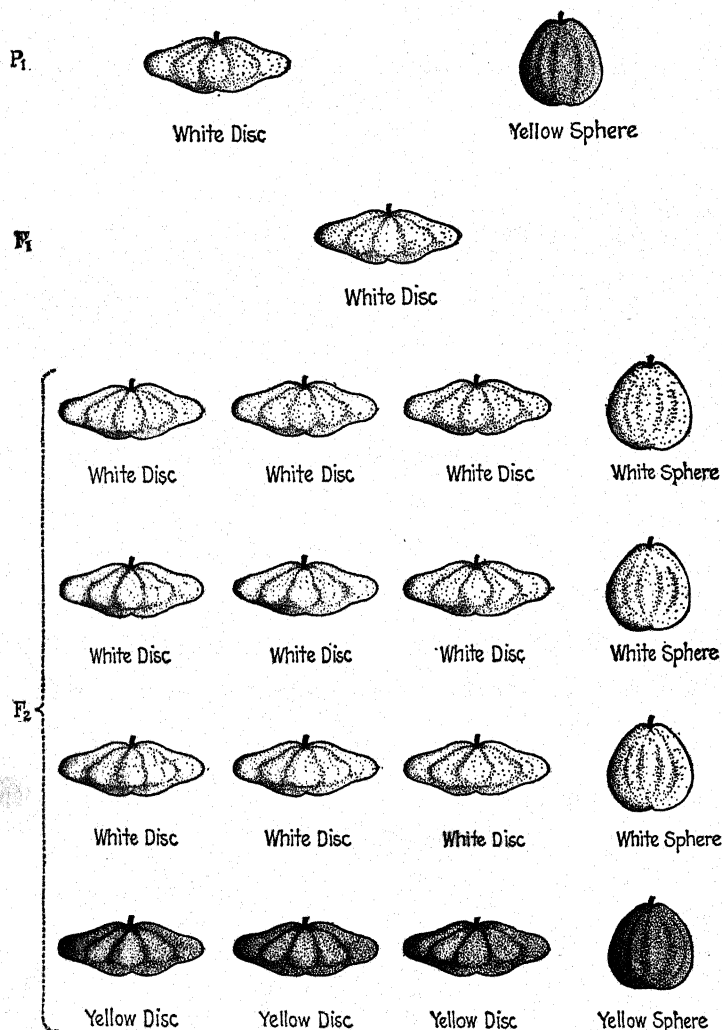


FIG. 26. The inheritance of two pairs of characters in summer squashes, illustrating Mendel's law of independent assortment. White is dominant over yellow and "disk" shape over "sphere." In F₂ there result $\frac{9}{16}$ white, disk; $\frac{3}{16}$ white, sphere; $\frac{3}{16}$ yellow, disk; and $\frac{1}{16}$ yellow, sphere plants.

already been noted that the gametes carry just *half* of the alleles of the parent individual, so that in this case one parent would produce gametes all of which carried RY ; and the other, gametes all of which carried ry ; and

the resulting F_1 hybrid offspring arising from a union of two of these gametes would consequently have the genotype $Rr Yy$. Now the crux of the problem, as in that of segregation, lies in the kinds of gametes produced by this F_1 individual. When the character of seed surface alone is considered, it is found that the $F_1 Rr$ individual produces gametes half of which carry R and half r . It is clear, however, that every gamete must necessarily contain within itself not only an allele for seed surface but one for seed color as well and, indeed, genes affecting every other character of the plant. Half of these same gametes must, therefore, contain allele Y and half the allele y ; but in any given gamete it seems to be *purely a matter of chance* as to whether the gene for round seeds is associated with that for yellow seeds or with that for green seeds. The particular combination of genes which enters the F_1 plant from each parent (round with yellow and wrinkled with green in this case) has no effect whatever upon the way in which they are associated in the gametes formed by this F_1 plant. *Their assortment is independent.* Of that half of the gametes which carry the gene for round seeds, a half in turn (or a quarter of the whole) carry yellow and a half carry green; and of that half which carry the gene for wrinkled seeds, a half also carry yellow and a half green. The F_1 hybrid may, therefore, be expected to produce four kinds of gametes in approximately equal numbers: RY , Ry , rY , and ry .

Now if two such F_1 plants, each of them producing four kinds of gametes, are crossed, there will obviously be 16 possible combinations among their gametes, for there will be four kinds of pollen grains and four kinds of egg cells. The union of these gametes in fertilization is here, too, a random one, any type of pollen grain being as likely to effect fertilization as any other; and any type of egg cell being as likely to be fertilized as any other, no selective preference being exhibited between them. The 16 possible combinations which appear among the F_2 offspring will, therefore, be equally numerous. The parents, F_1 , and F_2 of the cross which has been used as an example are represented diagrammatically in Fig. 27 as to both their genotypes and their appearance, the 16 squares in F_2 representing the 16 possible combinations of gametes. A count of these squares makes clear how the 9:3:3:1 ratio arises, for 9 out of these 16 individuals are in appearance round and yellow, 3 are round and green, 3 are wrinkled and yellow, and only 1 is wrinkled and green.

Difference between Genotype and Phenotype. It is obvious, however, that in the F_2 generation the 16 types will not all be *visibly* different, since some of the combinations will look alike, as dominance causes heterozygous individuals to look like homozygous dominant ones. As far as actual appearance goes, therefore, there will be only *four* kinds of individuals, and some of these groups will be much more numerous than others. There are,

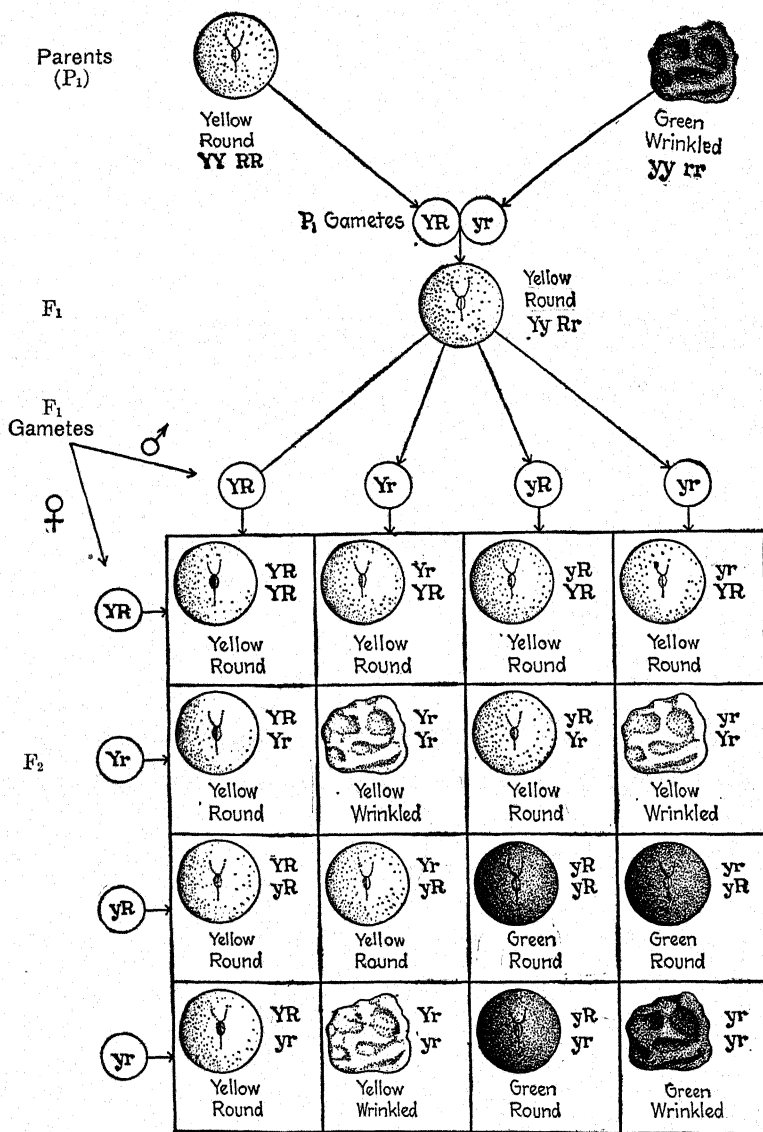


FIG. 27. Diagram showing the independent assortment in peas of two pairs of characters in which dominance is complete. In a cross between a plant homozygous for yellow and round seeds and a green, wrinkled-seeded one, the appearance, genotype, and gametes of parents and F₁ are shown. The results of random union between the four types of gametes formed by the F₁ heterozygote are presented in the F₂ checkerboard.

for example, four kinds of round-seeded and yellow-seeded individuals: those with the genotype $RR YY$, which are homozygous for both round and yellow and will breed true if inbred; those with the genotype $RR Yy$,

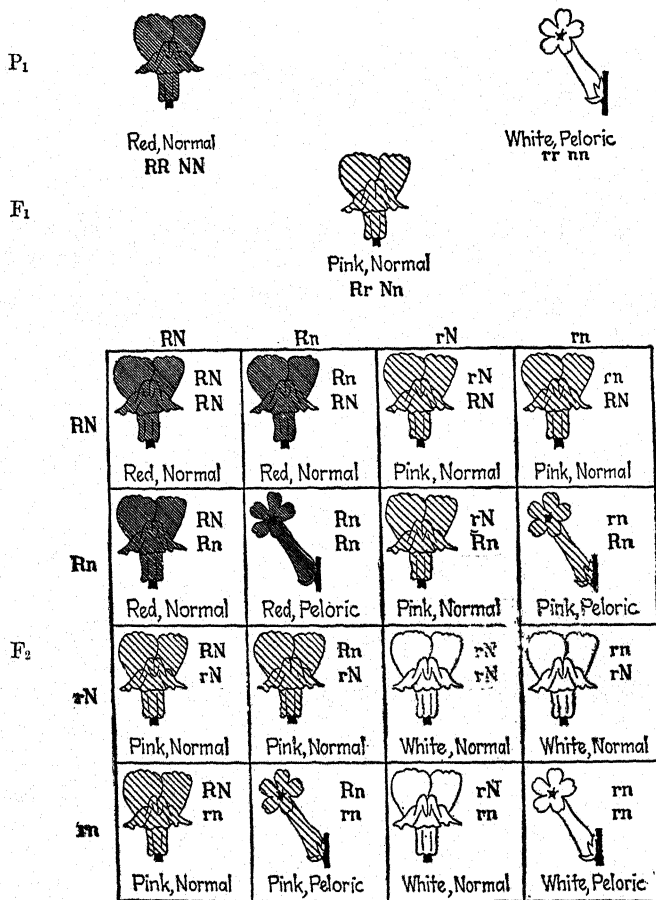


FIG. 28. Diagram showing the independent inheritance in snapdragons of two pairs of characters, in one of which dominance is complete and in the other of which it is lacking. In a cross between a plant homozygous for red flowers of normal shape and one with white and abnormal (peloric) flowers, the appearance and genotype of parents, F_1 , and F_2 are shown.

which are homozygous for round but heterozygous for yellow and will therefore breed true to round but not to yellow; those with the genotype $Rr YY$, which are heterozygous for round and homozygous for yellow and will breed true to yellow but not to round; and those with the genotype $Rr Yy$, which are heterozygous for both and will breed true to neither character

but will produce offspring which are exactly like the F_2 . A study of the squares in Fig. 27 shows that these four types should be found not in equal numbers but in the proportion of 1:2:2:4, respectively. Mendel tested this assumption experimentally and inbred all of his 315 F_2 plants which bore round and yellow seeds. He obtained offspring from 310 of them, of which 38 produced plants all bearing round and yellow seeds; 65 produced plants all bearing round seeds but some yellow and some green; 60 produced plants all bearing yellow seeds but some round and some wrinkled; and 138 produced plants of all four types.

Of course, it should be borne in mind that the characteristic 9:3:3:1 ratio is to be found only when both characters show complete dominance. If dominance is partial or absent, the heterozygous individuals are different in appearance from the pure ones and more than four F_2 groups will thus be visibly distinguishable. The results of a dihybrid cross in which one character pair shows complete dominance and the other does not are shown in Fig. 28. The presence or absence of dominance, however, has no bearing whatever on the fundamental fact of the independence of assortment of genes in the gametes.

Dihybrid Test Cross. The validity of the assumption that different genes assort independently can be submitted to a further test. The plants grown from the yellow-round seeds of the F_1 generation (Fig. 27) may be test-crossed (backcrossed) to the double recessive parent which produced green and wrinkled seeds. The F_1 hybrid plants carry, as we know, the genes $Yy Rr$, and since the parental genes assort independently, these plants form four kinds of gametes in equal numbers, namely, gametes with the genes YR , Yr , yR , and yr . The green-wrinkled parent forms only one kind of gamete, yr . The expected result in the progeny of the test cross may, then, be found in the lowermost line of squares of the checkerboard in Fig. 27. We should obtain yellow-round seeds (genotypically $Yy Rr$), yellow-wrinkled ($Yy rr$) green-round ($yy Rr$), and green-wrinkled ones ($yy rr$) in equal numbers, that is, in a ratio approaching $\frac{1}{4}:\frac{1}{4}:\frac{1}{4}:\frac{1}{4}$. This is what is actually obtained in experiments, which, accordingly, confirm Mendel's theory.

Similarly, one can predict the results to be obtained in *Drosophila* if the F_1 hybrids of gray-vestigial and ebony-long-winged flies (Fig. 25) are test-crossed to double recessive ebony-vestigial ones. The F_1 hybrids are phenotypically normal, that is, they have gray-body color and long wings, but they carry the genes for the ebony-body color and for vestigial wings in heterozygous condition. The progeny of the test cross will, then, consist of normal (gray and long-winged), gray-vestigial, ebony long-winged, and ebony-vestigial flies in a ratio approximately 1:1:1:1. Test crossing the F_1 hybrids in summer squashes (Fig. 26) to plants with yellow spherical

fruits will produce segregation in a ratio of 1 white, disk:1 yellow, disk:1 white, sphere:1 yellow, sphere. Finally, a test cross of the pink and normal-flowered snapdragons from the F_1 generation of the cross shown in Fig. 28 to white, peloric plants will produce a segregation in a ratio of 1 pink, normal:1 pink, peloric:1 white, normal:1 white, peloric plants.

The Trihybrid. When individuals differing in *three* independent characters are crossed, the situation is naturally more complex but the principle of independent assortment still holds good. If a homozygous round-seeded, yellow-seeded, and colored-flowered pea plant is crossed with a wrinkled-seeded, green-seeded, and white-flowered one, the F_1 hybrids are all, of course, round-seeded, yellow-seeded, and colored-flowered. Since the assortment of these three sets of genes in the gametes is independent, the F_1 plants will evidently produce *eight* kinds of gametes: one-half of one-half of one-half, or one-eighth, carrying the factors for round, yellow, and colored; one-eighth those for round, yellow, and white; one-eighth those for round, green, and colored; one-eighth those for round, green, and white; one-eighth those for wrinkled, yellow, and colored; one-eighth those for wrinkled, yellow, and white; one-eighth those for wrinkled, green, and colored; and one-eighth those for wrinkled, green, and white.

In the F_2 generation produced by random union among these eight kinds of gametes there will evidently be 64 equally possible and theoretically equally frequent combinations. These 64 F_2 types, with their appearance and their genotypic constitution, may be presented in a checkerboard which represents the results of a trihybrid cross. A study of such a group shows that there are only eight *visibly different* forms: $\frac{27}{64}$ (or $\frac{3}{4}$ of $\frac{3}{4}$ of $\frac{3}{4}$) have all three dominant characters; three groups each with $\frac{9}{64}$ (or $\frac{3}{4}$ of $\frac{3}{4}$ of $\frac{1}{4}$) show two of the dominants and one of the recessives; three groups each with $\frac{3}{64}$ (or $\frac{3}{4}$ of $\frac{1}{4}$ of $\frac{1}{4}$) show one dominant and two recessives; and only $\frac{1}{64}$ (or $\frac{1}{4}$ of $\frac{1}{4}$ of $\frac{1}{4}$) show all three recessive characters. The ratio of 27:9:9:9:3:3:3:1 is, therefore, typical for such a trihybrid, where all the characters show complete dominance.

As in the monohybrid and dihybrid crosses, many of these F_2 individuals which look alike are quite different genotypically, and will produce very different offspring when inbred (Table V). Of the $\frac{27}{64}$ which appear round, yellow, and colored, for example, there are eight kinds of plants, each of which will breed differently from the rest. Of course, where one or more of the characters studied show incomplete dominance, so that heterozygous individuals may be distinguished at sight from homozygous ones, the number of visibly different classes will be larger than eight and the ratio between them will be correspondingly altered.

In the same way, individuals differing in four characters may be brought together in a cross, and in such a case the F_2 is even more complicated than

TABLE V.—THE THEORETICAL NUMBER OF INDIVIDUALS, WITH THEIR GENOTYPES AND BREEDING BEHAVIOR, EXPECTED IN F₂ FROM A TRIHYBRID CROSS OF A ROUND, YELLOW-SEEDED, COLORED-FLOWERED VARIETY OF PEAS WITH A WRINKLED, GREEN-SEEDED, WHITE-FLOWERED ONE

Number of individuals	Genotype class	Phenotype class	Ratio of phenotypes	Breeding behavior when self-fertilized
1	$RR YY CC$	Round Yellow Colored	27	Breeds true
2	$Rr YY CC$			Segregates round-wrinkled, 3:1
2	$RR Yy CC$			Segregates yellow-green, 3:1
2	$RR YY Cc$			Segregates colored-white, 3:1
4	$Rr Yy CC$			Segregates round-wrinkled, yellow-green, 9:3:3:1
4	$Rr YY Cc$			Segregates round-wrinkled, colored-white, 9:3:3:1
4	$RR Yy Cc$			Segregates yellow-green, colored-white, 9:3:3:1
8	$Rr Yy Cc$			Segregates round-wrinkled, yellow-green, colored-white, 27:9:9:9:3:3:3:1
1	$RR YY cc$	Round Yellow White	9	Breeds true
2	$RR Yy cc$			Segregates yellow-green, 3:1
2	$Rr YY cc$			Segregates round-wrinkled, 3:1
4	$Rr Yy cc$			Segregates round-wrinkled, yellow-green, 9:3:3:1
1	$RR yy CC$	Round Green Colored	9	Breeds true
2	$RR Yy Cc$			Segregates colored-white, 3:1
2	$Rr yy CC$			Segregates round-wrinkled, 3:1
4	$Rr Yy Cc$			Segregates round-wrinkled, colored-white, 9:3:3:1
1	$rr YY CC$	Wrinkled Yellow Colored	9	Breeds true
2	$rr Yy CC$			Segregates yellow-green, 3:1
2	$rr YY Cc$			Segregates colored-white 3:1
4	$rr Yy Cc$			Segregates yellow-green, colored-white, 9:3:3:1
1	$rr yy CC$	Wrinkled Green Colored	3	Breeds true
2	$rr yy Cc$			Segregates colored-white 3:1
1	$rr YY cc$	Wrinkled Yellow White	3	Breeds true
2	$rr Yy cc$			Segregates yellow-green, 3:1
1	$RR yy cc$	Round Green White	3	Breeds true
2	$Rr yy cc$			Segregates round-wrinkled, 3:1
1	$rr yy cc$	Wrinkled Green White	1	Breeds true

that of a trihybrid, 16 kinds of gametes being produced by the F_1 and 256 possible combinations resulting in the F_2 .

In practice, when the parents crossed differ in several genes, test crosses of F_1 hybrids to multiple recessive types are easier to analyze than are F_2 segregations. This is simply because the numbers of possible combinations of gametes in an F_2 progeny are equal to the squares of the numbers of combinations observed in test crosses. For example, the trihybrid with round and yellow seeds and colored flowers ($Rr Yy Cc$, Table V), when test-crossed to plants with wrinkled, green seeds and white flowers ($rr yy cc$) will produce 8 combinations, instead of the 64 combinations observed in F_2 . The segregation observed in the progeny of the test cross will consist of approximately equal numbers of the following plants:

Round, yellow, colored ($Rr Yy Cc$); wrinkled, green, colored ($rr yy Cc$).
 Round, yellow, white ($Rr Yy cc$); wrinkled, yellow, white ($rr Yy cc$)
 Round, green, colored ($Rr yy Cc$); round, green, white ($Rr yy cc$)
 Wrinkled, yellow, colored ($rr Yy Cc$); wrinkled, green, white ($rr yy cc$)

Table VI gives the results observed in a test cross involving four pairs of genes in rats. Animals were obtained which had dense and solid agouti-

TABLE VI.—INDEPENDENT SEGREGATION OF FOUR PAIRS OF ALLELES IN THE LABORATORY RAT

A = agouti pattern, a = nonagouti; H = solid-colored, h = white hooded; R = dark eye, r = ruby eye; D = dense color, d = dilute color

[Data from Roberts, Dawson, and Madden in *Biometrika* 31 (No. 9): 56-66]

Mating of $Aa Hh Rr Dd \times aa hh rr dd$

Phenotypes*	Numbers of offspring
$A H R D$	41
$A H R d$	36
$A H r D$	41
$A h R D$	29
$A H r d$	39
$A h R d$	33
$A h r D$	35
$A h r d$	25
$a H R D$	50
$a H R d$	29
$a H r D$	38
$a h R D$	30
$a H r d$	34
$a h R d$	34
$a h r D$	32
$a h r d$	30

* Since A , H , R , and D are fully dominant to their alleles, these phenotypes can be specified by the dominant allele.

colored fur and dark eyes but which were heterozygous for the recessive genes giving dilute-colored, white-hooded (spotted), and nonagouti fur and ruby-colored eyes. The genotype of such animals can be written $Aa Hh Rr Dd$, and they are expected to produce 16 kinds of gametes in equal numbers, containing all possible combinations of the four genes. Such animals were backcrossed to quadruple recessive, dilute, nonagouti, white-hooded, and ruby-eyed animals, the genotype of which was evidently $aa hh rr dd$ and which produced gametes of only one kind, with the genes $ah rd$. The expected result of the backcross is the appearance of 16 classes of animals, listed in Table VI, in equal numbers, and showing all combinations of the parental traits.

Polyhybrids. The situations obtained in the offspring of hybrids heterozygous for many genes (polyhybrids) may be quite complex. As the number of genes involved in a given cross increases, the number of possible gene combinations increases rapidly. Every added gene multiplies the number of different classes of gametes formed by 2, the number of genotypes produced in the F_2 generation by 3, and the number of possible combinations of gametes in F_2 by 4. A simple mathematical expression of these relations is found in Table VII.

TABLE VII.—THE RELATION BETWEEN THE NUMBER OF PAIRS OF ALLELES INVOLVED IN A CROSS AND THE NUMBER OF PHENOTYPIC AND GENOTYPIC CLASSES IN F_2

Number of pairs of alleles involved in the cross	Number of visibly different F_2 classes of individuals if dominance is complete	Number of different kinds of gametes formed by the F_1 hybrid	Number of genotypically different combinations	Number of possible combinations of F_1 gametes
1	2	2	3	4
2	4	4	9	16
3	8	8	27	64
4	16	16	81	256
n	2^n	2^n	3^n	4^n

Furthermore, as the number of genes involved increases, the chance of recovering one of the original parent types in the F_2 grows rapidly less. When a single pair of alleles is involved, 1 in 4 of the F_2 will resemble one of the original parents in appearance and genotype; when two pairs are involved, 1 in 16; when three, 1 in 64; when four, 1 in 256; and so on. The generalized formula for determining the number of visibly different classes, of different kinds of gametes, of genotypically different combinations, and of possible combinations of F_1 gametes in crosses involving a known number of pairs of alleles is shown in the last line of Table VII.

A study of the assortment and recombination of genes which go on in the hybrids when more than one allele pair is involved makes it clear how

readily new character combinations are formed and emphasizes the importance of hybridization as a cause of increased variation. A thorough understanding of the principles which are concerned in this process renders it easy to control and to predict the appearance of new types of animals and plants and is one of the chief contributions which the science of genetics has made to the art of practical breeding.

It is easy to see that, if a parent is heterozygous for many genes, the chance that any two gametes produced by this parent will contain the same gene complement rapidly diminishes. Suppose that an individual is heterozygous for only 20 genes; the number of kinds of gametes with different gene complements which such an individual can produce will be $2^{20} = 1,073,741,824$. The probability that any two children will inherit the same gene complement from either of their parents is, therefore, small. If both parents are heterozygous for 20 genes each, the number of genotypes potentially possible in their offspring is 4^{20} , which is an enormous number indeed. In practice, there is no chance that any two children of such parents will possess the same genotype unless, of course, they are identical twins, which arise from a single zygote (*cf.* p. 20).

These calculations assume, of course, that Mendel's second law, the law of independent assortment, is always valid. Actually, studies carried on since the rediscovery of Mendel's work in 1900 have shown that only those genes which are located in different chromosomes are assorted independently. When two or more genes lie in the same chromosome, the phenomena of *linkage* are observed, which limit the applicability of the second law of Mendel. These matters will be discussed in detail in later chapters.

The "Chi-square" Method for Testing Goodness of Fit. Where a segregating population falls into three or more classes, it is impossible to determine how closely it fits a given theoretical expectation by using the method, described in the previous chapter, of comparing the deviation with its standard error, for there may be several deviations. Instead, biometricians have measured goodness of fit by adding together the proportional deviations of each class, obtaining a constant known as χ^2 (chi square), and from this determining the probability that a deviation as great or greater will occur by chance. χ^2 is obtained by squaring the deviation of each class from the theoretical expectation for that class, dividing this by the theoretical expectation for that class, and adding together the results from all classes. By means of a table originally calculated by Elderton and presented in a more simplified form by Fisher (Table VIII), it is possible to obtain for a given value of χ^2 and a given number of classes the value of P , which measures the probability that a deviation as great or greater will occur by chance, in other words, the percentage of cases in which such a deviation may be expected by chance.

If, for example, in a given F_2 population where segregation in the proportions of $\frac{9}{16} AB$, $\frac{3}{16} Ab$, $\frac{3}{16} aB$, and $\frac{1}{16} ab$ is expected, there actually occur 456 AB , 155 Ab , 141 aB , and 48 ab , the theoretical expectation would evidently be 450 AB , 150 Ab , 150 aB , and 50 ab . The derivation of χ^2 for this population and ratio is as follows:

	AB	Ab	aB	ab
Actual numbers	456	155	141	48
Theoretical expectation on 9:3:3:1 (e)	450	150	150	50
Deviation from expectation (d)	6	5	9	2
d^2	36	25	81	4
d^2/e080	.166	.540	.080
Total (sum of d^2/e) = .866 = χ^2 $N' = 3$				

Consultation of Table VIII shows that in a population of four classes a value for χ^2 of .866 means that P will have a value of between .80 and .90, in other words, that in 80 to 90 per cent of similar cases as great or greater deviation from a 9:3:3:1 ratio would be found, and that the present population therefore fits that ratio very well.

In an F_2 population in which independent assortment was expected between flower color and pollen shape in the sweet pea, Bateson and Punnett found the following distribution:

	Purple, long	Purple, round	Red, long	Red, round
Found	226	95	97	1
Expected (9:3:3:1)	235.69	78.56	78.56	26.19
Deviation (d)	9.69	16.44	18.44	-25.19
d^2/e40	3.44	4.29	24.23
$\chi^2 = \Sigma d^2/e = 32.36$ $N' = 3$ $P = <.01$				

The value of χ^2 with $N' = 3$ corresponds to a value of P far below .01 (Table VIII). Consequently we can say that the chances of a fit of observed to calculated data as bad as or worse than this are considerably less than 1 per cent. The departure of observation from theory is not due to chance; one is thus led to doubt the theory, in this case, independent assortment. These data constituted, in fact, the first significant exception to the principle of independent assortment and were shown to be due to dependent assortment or linkage (*cf.* Chap. IX).

It will be noted that in Table VIII the value of N' , designating the "degree of freedom," is always one less than the number of segregating

TABLE VIII.—VALUES OF P (TOP LINE) FOR VARIOUS VALUES OF CHI SQUARE (VERTICAL COLUMNS) AND FOR VARIOUS DEGREES OF FREEDOM (N')

The degrees of freedom are one less than the number of classes

(From R. A. Fisher, "Statistical Methods for Research Workers," by permission of author and publishers, Messrs. Oliver and Boyd)

N'	$P = .99$.98	.95	.90	.80	.70	.50	.30	.20	.10	.05	.02	.01
1	.00016	.00063	.0039	.016	.064	.148	.455	1.074	1.642	2.706	3.841	5.412	6.635
2	.0201	.0404	.103	.211	.446	.713	1.386	2.408	3.219	4.605	5.991	7.824	9.210
3	.115	.185	.352	.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	9.837	11.341
4	.297	.429	.711	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	11.668	13.277
5	.554	.752	1.145	1.610	2.343	3.000	4.351	6.064	7.289	9.236	11.070	13.388	15.086
6	.872	1.134	1.635	2.204	3.070	3.828	5.348	7.231	8.558	10.645	12.592	15.033	16.812
7	1.239	1.564	2.167	2.833	3.822	4.671	6.346	8.383	9.803	12.017	14.067	16.622	18.475
8	1.646	2.032	2.733	3.490	4.594	5.527	7.344	9.524	11.030	13.362	15.507	18.168	20.090
9	2.088	2.532	3.325	4.168	5.380	6.393	8.343	10.656	12.242	14.684	16.919	19.679	21.666
10	2.558	3.059	3.940	4.865	6.179	7.267	9.342	11.781	13.442	15.987	18.307	21.161	23.209

classes. This is due to the fact that in calculations of this sort, where a series of classes are involved, the size of the final class cannot be a matter of chance, since it must include everything that is left over. Its size is thus already fixed, and it is not "free" to vary as the others are. In cases like the present one (and most genetic problems) the number of classes is always one more than the degrees of freedom, but in other biometrical problems there may be a greater difference, and the table is therefore more generally useful if the degrees of freedom rather than the class numbers are stated.

The χ^2 method can, of course, be used to measure goodness of fit where there are only two classes, instead of the method of the standard error of the ratio as previously described. It may be employed in all sorts of modified ratios and is of much value in determining which ratio a given segregating population fits best; for this is the one which will give the smallest value of χ^2 , and thus the largest value for P , when the ratio of the actual population is compared with it.

References will be found at the end of Chap. II.

PROBLEMS

Note. In the summer squash, white fruit W , is dominant over yellow, w ; and "disk" fruit shape D is dominant over "sphere" shape d .

64. In a cross between a squash plant homozygous for yellow fruit color and disk fruit shape and one homozygous for white fruit color and sphere fruit shape, what will be the appearance, as to color and shape of fruit, of the F_1 ? of the F_2 ? of the offspring of a cross of the F_1 with the yellow, disk parent? with the white, sphere parent?

✓ 65. What are the gametes formed by the following squash plants, the genotypes of which for fruit color and shape are given; and what will be the appearance of the offspring from each cross:

$WW dd \times ww DD$

$Ww Dd \times Ww dd$

$Ww DD \times ww dd$

$Ww Dd \times ww dd$

$Ww Dd \times Ww DD$

$Ww Dd \times Ww Dd$

Note. In the following six questions, all of which deal with fruit color and shape in summer squash, the appearance of parents and offspring is stated. Determine in each case the genotypes of the parents.

66. White, disk crossed with yellow, sphere gives one-half white, disk and one-half white, sphere.

67. White, sphere crossed with white, sphere gives three-fourths white, sphere and one-fourth yellow, sphere.

68. White, disk crossed with yellow, sphere gives one-fourth white, disk; one-fourth white, sphere; one-fourth yellow, disk; and one-fourth yellow sphere.

69. White, disk crossed with white, sphere gives three-eighths white, disk; three-eighths white, sphere; one-eighth yellow, disk; and one-eighth yellow, sphere.

70. Yellow, disk crossed with white, sphere gives all white, disks.

✓ 71. White, disk crossed with white, disk gives 28 white, disk plants; 9 white, sphere plants; 10 yellow, disk plants; and 3 yellow, sphere plants.

Note. In guinea pigs, rough coat *R* is dominant over smooth coat, *r*; and black coat *B* is dominant over white *b*.

72. Cross a homozygous rough, black animal with a smooth, white one. What will be the appearance of the F_1 ? of the F_2 ? of the offspring of a cross of the F_1 back with the rough, black parent? with the smooth, white one?

73. In the F_2 generation in the preceding question, what proportion of the rough, black individuals may be expected to be homozygous for both characters?

74. A rough, black guinea pig bred with a rough, white one gives 28 rough, black; 31 rough, white; 11 smooth, black; and 9 smooth, white. What are the genotypes of the parents?

75. Two rough, black guinea pigs when bred together have two offspring, one of them rough, white and the other smooth, black. If these same parents were to be bred together further, what offspring would you expect from them?

Note. In poultry, feathered legs *F* are dominant over clean legs *f*; and pea comb *P*, over single comb, *p*.

76. Two cocks A and B are bred to two hens C and D. All four birds are feathered-legged and pea-combed. Cock A with both hens produces offspring which are all feathered and pea. Cock B with hen C produces both feathered and clean but all pea-combed; but with hen D he produces all feathered but part pea-combed and part single. What are the genotypes of these four birds?

77. The offspring of a feathered-legged, pea-combed cock bred to a clean-legged, pea-combed hen are all feathered-legged. Most of them are pea-combed, but some singles appear among them. What are the genotypes of the parents? What would be the offspring expected from a cross of this hen with one of her feathered-legged, single-combed male offspring?

78. In swine, white coat is dominant over black and the "mule-footed" condition over that with normal feet. A white, mule-footed boar, A, always produces white, mule-footed offspring, no matter to what sow he is bred. Another boar, B, however, also white and mule-footed, when bred to black sows produces about half white and half black offspring, and when bred to normal-footed sows, about half mule-footed and half normal offspring. Explain this difference between these two animals by comparing their genotypes for these two traits.

Note. In man assume that brown eyes *B*, are dominant over blue, *b*, and right-handedness, *R*, over left-handedness, *r*.

✓ 79. A right-handed, blue-eyed man whose father was left-handed marries a left-handed, brown-eyed woman from a family in which all the members have been brown-eyed for several generations. What offspring may be expected from this marriage as to the two traits mentioned?

80. A brown-eyed, right-handed man marries a blue-eyed, right-handed woman. Their first child is blue-eyed and left-handed. If other children are born to this couple, what will probably be their appearance as to these two traits?

81. A right-handed, blue-eyed man marries a right-handed, brown-eyed woman. They have two children, one left-handed and brown-eyed and the other right-handed and blue-eyed. ' By a later marriage with another woman who is also right-handed and brown-eyed, this man has nine children, all of whom are right-handed and brown-eyed. What are the genotypes of this man and his two wives?

Note. In cattle the polled condition, P , is dominant over the horned, p ; and in Shorthorns the heterozygous condition of red coat, R , and white coat, r is roan.

82. If a homozygous polled, white animal is bred to a horned, red one, what will be the appearance of the F_1 ? of the F_2 ? of the offspring of a cross of the F_1 with the polled, white parent? with the horned, red parent?

83. A polled, roan bull bred to a horned, white cow produces a horned, roan daughter. If this daughter is bred to her father, what offspring may be expected as to horns and coat color?

Note. In snapdragons red flower color, R , is incompletely dominant over white, r , the heterozygous condition being *pink*; and normal, broad leaves, B , are incompletely dominant over narrow, grasslike ones, b , the heterozygous condition being intermediate in leaf breadth.

84. If a red-flowered, broad-leaved plant is crossed with a white-flowered, narrow-leaved one, what will be the appearance of the F_1 and the F_2 ?

Note. In garden peas, tall vine (T) is dominant over dwarf (t), green pods (G) over yellow (g), and round seed (R) over wrinkled seed (r).

85. If a homozygous dwarf, green, wrinkled pea plant is crossed with a homozygous tall, yellow, round one, what will be the appearance of the F_1 ? What gametes does the F_1 form? What is the appearance of the F_2 ? What is the appearance of the offspring of a cross of the F_1 with its dwarf, green, wrinkled parent? with its tall, yellow, round parent?

- 86. What will be the appearance of the offspring of the following crosses, in which the genotypes of the parents are given:

$$TT\ Gg\ Rr \times tt\ Gg\ rr$$

$$Tt\ GG\ Rr \times Tt\ Gg\ Rr$$

$$tt\ gg\ Rr \times Tt\ Gg\ rr$$

$$Tt\ Gg\ rr \times tt\ Gg\ Rr$$

Note. In the following four questions, all of which concern garden peas, find the genotypes of the parents as to vine height, pod color, and seed shape:

87. A tall, yellow, round plant crossed with a dwarf, green, round one produces offspring three-eighths of which are tall, green, and round; three-eighths dwarf, green, and round; one-eighth tall, green, and wrinkled; and one-eighth dwarf, green, and wrinkled.

88. A tall, green, wrinkled plant crossed with a dwarf, green, round one produces offspring three-fourths of which are tall, green, and round and one-fourth of which are tall, yellow, and round.

89. A tall, green, round plant crossed with a tall, yellow, round one produces 26 tall, green, round offspring; 10 tall, green, wrinkled; 9 dwarf, green, round; and 3 dwarf, green, wrinkled.

90. A tall, yellow, round plant crossed with a dwarf, green, round one produces 58 tall, green, round offspring; 61 tall, yellow, round ones; 62 dwarf, green, round ones; 59 dwarf, yellow, round ones; 19 tall, green, wrinkled ones; 20 tall, yellow, wrinkled ones; 21 dwarf, green, wrinkled ones; and 20 dwarf, yellow, wrinkled ones.

91. In tomatoes, red fruit is dominant over yellow, two-loculed fruit over many-loculed, and tall vine over dwarf. A breeder has pure races of red, two-loculed, dwarf plants and of yellow, many-loculed, tall ones. He wants a race of red, many-loculed, tall plants. If he crosses his two races and raises an F_1 and an F_2 , what proportion of this F_2 will be, in appearance, the type he desires? What proportion of these will be homozygous for all three characters? How can he determine which are the homozygous plants?

92. In poultry, the white plumage of Leghorns is dominant over colored plumage, feathered shanks over clean, and pea comb over single. If a homozygous white, feathered, pea bird is crossed with a colored, clean, single one, what proportion of the white, feathered, pea birds in the F_2 from this cross will prove to be homozygous if mated to colored, clean, single birds?

93. In snapdragons normal flowers are dominant over peloric ones and tallness over dwarfness. Red flower color is incompletely dominant over white, the heterozygous condition being pink. If a homozygous red, tall, normal-flowered plant is crossed with a homozygous, white, dwarf, peloric-flowered one, what proportion of the F_2 will resemble the F_1 in appearance?

94. If one individual is homozygous for four dominant factors and another for their four recessive alleles and if these two individuals are crossed, what proportion of the F_2 from this cross will resemble each parent, respectively, in appearance?

95. By finding the values of χ^2 and P , determine how closely each of the five following F_2 populations fits a 9:3:3:1 ratio. Which are to be regarded as examples of this ratio, and which are not?

<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>
315	108	101	32
51	11	16	2
860	315	340	117
75	35	41	9
1,770	610	618	202

96. Determine the goodness of fit of the following F_2 population to a 3:1 ratio using both the standard-error and the χ^2 methods. Which do you think is the more satisfactory method to use in such a case and why?

<i>A</i>	<i>a</i>
1,182	418

97. Make the same assumptions as in Problem 52 except that the heterozygous invaders are *Cc Dd*. What will be the relative frequency in the fifth generation of the four phenotypes expected from random mating?

98. In a human population breeding at random an albino individual appears about once in 20,000 births. Taste-blind persons appear about three times per 10 births. If the genes for taste blindness and albinism show independent assortment in the same population, what should be the frequency of taste-blind albinos?

99. In Problem 98 what fraction of the population would you expect to be heterozygous for both albinism and taste blindness?

100. Calculate the values of χ^2 and P for the data in Table VI. How frequently might such a deviation from the expected ratio be encountered?

101. Calculate the chance that each of the seven genes studies by Mendel should belong to a different one of the seven linkage groups found in peas.

CHAPTER IV

ALLELISM

Mendel's great contribution to genetics was the demonstration that the development of an organism is directed by an aggregation of separable units called genes which are transmitted from parents to offspring. An individual may receive from its parents two unlike genes for some trait. For example, a pea plant may inherit a gene for red flowers from one parent and a gene for white flowers from the other. In such a heterozygote, or hybrid, the unlike genes do not fuse or mix but segregate cleanly when the hybrid forms its gametes. Further, the maternal and paternal genes for different traits do not stay together in the offspring of the hybrid, but enter into all combinations in the gametes.

We learn about the existence of genes for various traits from observations on segregation and recombination of characters in the offspring of heterozygotes. The behavior of flower color in crosses between red and white varieties of peas is the evidence that in peas there is a gene for flower color. If all pea plants were red-flowered or all were white, one could not observe segregation in flower color in hybrids and consequently would not know that a gene for flower color existed. Similarly, if all men were brown-eyed or all blue-eyed, no eye-color genes would be known in man. Now, genetically different flower colors are found in peas because a gene which influences flower color had at some time in the past changed by mutation, so that two variants, or alleles, of this gene now exist. Similarly, somewhere in the ancestry of the human species an eye-color gene underwent a change, giving rise to the allele for blue and the allele for brown eyes. Only those genes which have mutated to give rise to at least two distinguishably different alleles are known to us. Genes which have not mutated and genes that have changed but of which only one allele survived are not detected.

The existence of *allelism*, that is, of alternative states of the same gene having different phenotypic effects, is thus the condition which made Mendel's discovery possible and is a fact of primary importance in genetics. Since alleles can be detected only by their effects, it is important to know what kinds of effects a gene is capable of producing, what the relative degree of effect or dominance of each allele is, and how the several alternative conditions, or *multiple alleles*, of certain genes are related in hereditary transmission and in phenotypic effect.

Manifold Effects of Genes. The usual way of referring to a gene by its most marked effect on some one trait is apt to be misleading unless it is understood that this habitual form of speech is retained only because it is short and convenient. It does not imply any such one-to-one relation between a "character" and a gene or other hereditary particle as was assumed in Darwin's (1868) hypothesis of pangenesis or Weismann's (1892) determinant theory. In both these speculative hypotheses, elementary particles were supposed to be transmitted through the gametes as representatives of each character and organ of the body, for whose development they were responsible. The effect of one of the first genes studied by Mendel in peas, however, had to be interpreted in a very different way. We speak of this now as a gene for red flowers as opposed to colorless ones. Yet Mendel, who first identified it, referred to it by its effect on the color of the seed coats, which are gray or brown, and noted at the same time that the same gene was responsible also for reddish spots in the axils of the leaves and for development of color in the flowers (cf. Fig. 14, p. 34).

Similarly, the inheritance of wing length in crosses of normal and vestigial-winged vinegar flies (Fig. 29) is described by saying that the dominant normal allele of the "vestigial gene" produces long wings and the recessive allele gives rise to vestigial wings. This gene may even be called a "wing gene." Indeed, the change which first strikes our eye when the recessive allele is substituted for the dominant one is the reduction in size of wings. Yet when normal and vestigial flies are carefully compared, many differences come to light. Apart from short wings, vestigial flies have also modified balancers (halteres), the bristles of a certain pair on the dorsal side of the fly are erect instead of horizontal; the shape of the spermatheca is changed; the number of egg strings in the ovaries is decreased compared to normal when the vestigial larvae are well fed but relatively increased when poorly fed; length of life and fecundity are lowered; and there are still other differences.

When an allele or alleles cause changes in two or more not obviously related parts or characters, the gene is called a *pleiotropic* one and is said to have *multiple*, or *manifold*, effects. The "vestigial gene" might just as well be called a "bristle gene" or a "fecundity gene" as a "wing gene." It is

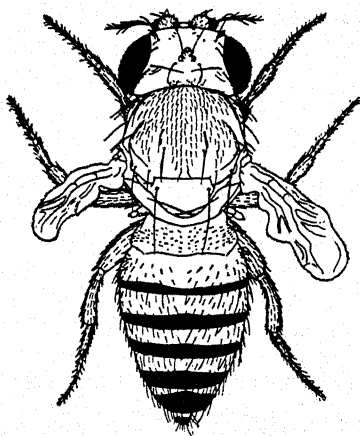


FIG. 29. Vestigial, a recessive mutation in *Drosophila melanogaster* with pleiotropic effects. (After Morgan.)

probable that careful studies would show that many, perhaps all, genes are pleiotropic although as a rule one trait produced by a gene change appears more striking than others. It must also be remembered that when red-flowered peas, homozygous for the dominant allele C , are compared with white-flowered ones, cc , the comparison discloses only the changes in the phenotype due to the *difference* between C and c . In other words, such a comparison does not necessarily tell us what the sum total of effects of either C or c is upon the organism. In order to find out all the effects of these genes one would have to compare CC and cc plants with others in which neither C nor c is present, which carry no allele of C at all. Such physical absences of a gene or genes are called *deficiencies* and will be discussed in a later chapter. In most cases, they lead to the death of the organism and thus act as *lethals* (cf. p. 98).

A single gene may thus be very important in the development of the organism and may modify many traits. As will be shown in Chapters V and VI, the converse proposition is also true since many, or even all, traits are determined by cooperation of several or many genes.

These considerations serve to emphasize the conclusion that the real unit of inheritance is not the developed character, which is visible and measurable and which, as has been seen, may be variable and complex, but the underlying unit, which is the gene. Not only is the organism formed under the cooperative influence of a large number of genes, but each gene, itself a distinct unit, exerts a widespread influence on many parts of the organism. An extension of this view may be seen in the idea that many fundamental characters of the organism are determined by genes acting on the whole animal or plant rather than on single parts. The mechanism by which genes may produce such effects is discussed in a later chapter (Chap. XVI).

Dominance. It happened that in the pea hybrids studied by Mendel the effects of one allele of a gene were always completely dominant to those of the alternative allele (red flower color dominant over white, round seeds dominant over wrinkled, etc.). Cases in which dominance is absent were, however, discovered very early in the history of genetic studies, as shown in the examples of Andalusian fowls and hybrids of red and white snapdragons (Chap. II).

All intermediate conditions between complete dominance and no dominance at all also occur. For example, the dominance of round over wrinkled in peas seemed to Mendel to be complete, yet later on microscopic examination showed that the starch grains of the hybrid are intermediate in some respects between those of the parent types. A fatal anemia in man (thalassemia major, or Mediterranean anemia) was supposed to be due to homozygosis for a gene which was completely recessive. Yet it has been

shown that persons heterozygous for this gene can be detected by examination of their blood, in which the red cells have characteristic but slight abnormalities resulting in a mild anemia (thalassemia minor) which is not usually considered to be a pathological condition.

Many cases are known in which a gene has dominant effects on some traits but recessive ones on others. This is often the case with the group of lethal genes discussed in the following chapter. The first instance of this kind was the allele in the house mouse, which has a recessive effect on viability, all homozygotes dying before birth, and a dominant effect on the coat color, which is yellow instead of gray (*cf.* p. 98).

Modification of Dominance. Dominance is not an invariable property of any allele of a gene. It sometimes can be affected by environmental influences, as well as by physiological factors such as age, sex, and other conditions. In the Jimson weed (*Datura*) purple stem color is completely dominant over green if the heterozygous plants are grown out of doors in the summer. In the greenhouse in winter the heterozygotes are distinguishable from the homozygous purple plants by paler color. The hybrids between a red- and an ivory-flowered snapdragon may be red if grown in bright light and at a low temperature; ivory, if kept in a shaded warm place; or intermediate in color, under intermediate conditions. In crosses between certain horned and hornless breeds of sheep, males heterozygous for a gene for hornlessness are horned, while females of the same genetic constitution are hornless. Hornlessness may therefore be said to be recessive in males but dominant in females. A gene for pattern baldness in human beings appears to be dominant in men, recessive in women (Fig. 30).

Occasionally a gene may appear to have a recessive effect in young heterozygotes but a dominant one at some later stage. The difference between left (sinistral) and right (dextral) coiling in snails appears to depend chiefly on a single pair of genes. The heterozygote may be either dextral or sinistral depending on the genotype of the mother for this trait, since the direction of coiling is maternally determined. Such heterozygotes, however, produce only dextral offspring, thus proving that the dextral condition is dominant although the dominant effect has here been delayed for a whole generation (see Chap. XVII).

The dominance relations between two alleles also depend upon other genetic factors. Thus the gene for forked bristles in *Drosophila* ordinarily behaves as a recessive but in the presence of one other independent gene may act as a partial dominant, while a gene for white spotting in mice may behave as a dominant in combination with certain other genes or as a recessive in combination with others (p. 131).

It sometimes happens that an allele which in one species is completely recessive becomes partly dominant when transferred to another species.

This effect is due to certain other genes which modify the dominance relations between the alleles in question (Fig. 31).

Isoalleles. It should not be assumed as a matter of course that the dominant or "normal" or "wild-type" allele is always and everywhere the same. Because of the limitations mentioned above, we generally cannot know whether the "red allele" in peas is the same in all varieties. We only know that it shows a similar dominance to white. Both Timoféeff-Resovsky and Muller found that wild-type *Drosophila* from different natural populations had different dominant (red-eye) alleles of the gene "white

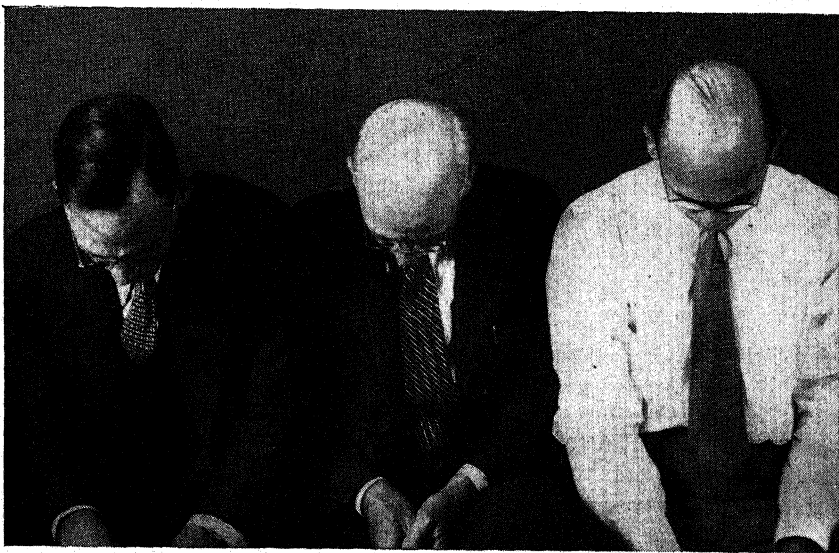


FIG. 30. Segregation of pattern baldness in the two sons (left and right) of a father with pattern baldness (center). (From L. H. Snyder's *Principles of Heredity*, Courtesy of D. C. Heath and Co.)

eye", as judged by their stability or by their different effects in combination. Stern found three different wild-type alleles of another *Drosophila* mutant, *cubitus interruptus*, which showed different degrees of dominance over the same mutant allele. He called such genes *isoalleles* because they are alike in their homozygous effects and their differences appear only in special combinations. Many such isoalleles probably escape detection.

Multiple Alleles. The examples discussed in this and preceding chapters have involved the segregation and relative expression of only two alternative conditions, or alleles, of each gene. However, it has been found that many and possibly all genes are able to change in a variety of ways and to give rise to several alternative variants, or multiple alleles. Thus a gene

A may change or mutate not merely to *a* but to other stable alleles such as $a^1, a^2, \dots a^n$. Although the relation between alleles which are members of a multiple series does not differ in principle from that which we found to hold for members of a simple pair of alleles, some additional methods and

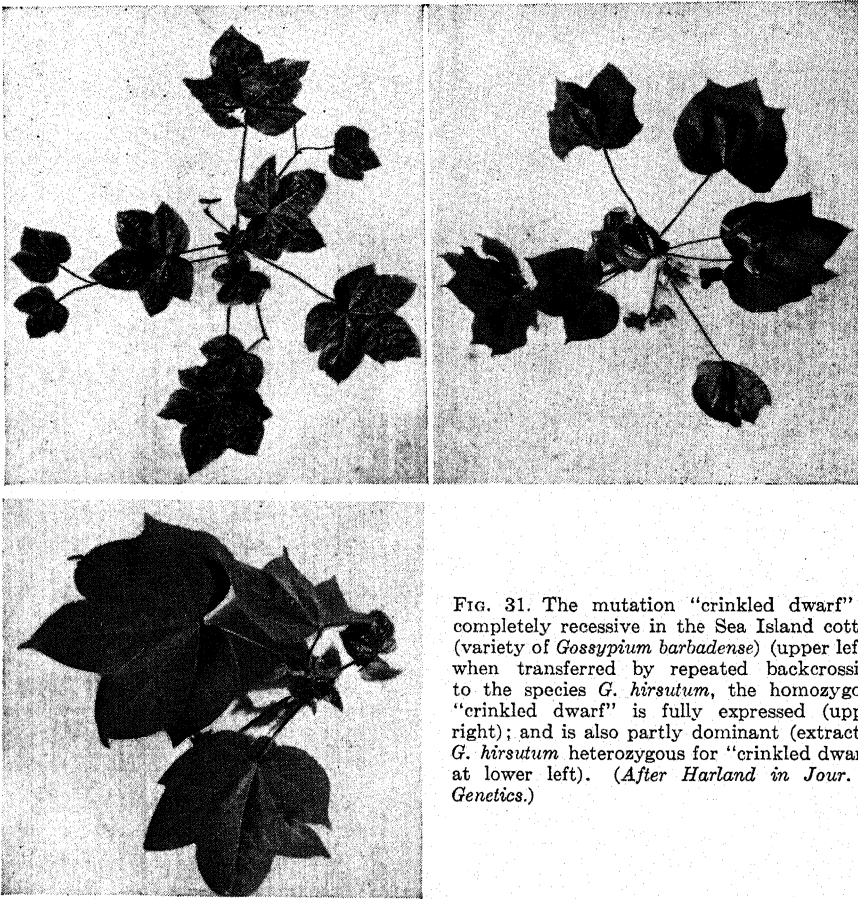


FIG. 31. The mutation "crinkled dwarf" is completely recessive in the Sea Island cotton (variety of *Gossypium barbadense*) (upper left); when transferred by repeated backcrossing to the species *G. hirsutum*, the homozygous "crinkled dwarf" is fully expressed (upper right); and is also partly dominant (extracted *G. hirsutum* heterozygous for "crinkled dwarf" at lower left). (After Harland in *Jour. of Genetics*.)

ideas are needed for detecting multiple alleles, for representing the greater variety of genotypes which they make possible, and for studying the phenotypic relationships among the alleles within a series.

The common pink-eyed white (albino) rabbit (Fig. 32), has long been known to act as a simple recessive to the colored type (Fig. 32). Crosses of colored with albino rabbits produce only colored progeny, which when inbred, produce colored and albino young in the ratio of $\frac{3}{4}$ colored: $\frac{1}{4}$

albino. Color, C , and albinism, c^a , thus form a pair of alleles. There is another form of albinism in rabbits known as Himalayan albinism (Fig. 32, center). Himalayans have pink eyes, and their fur is white except for

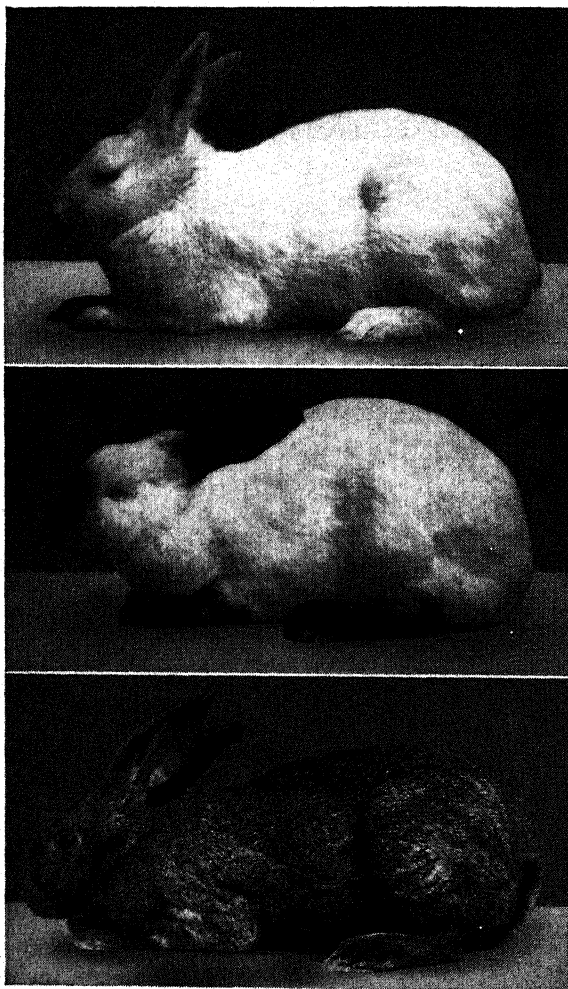


FIG. 32. Three alleles of a gene for coat color in rabbits (bottom fully colored, $C^+ C^+$; center, Himalayan albinism, $c^h c^h$; top, complete albinism, $c^a c^a$). (From Castle.)

the feet, tail, ears, and tip of the nose, which are black or dark brown. When these are crossed with fully colored rabbits, the F_1 is colored and in the F_2 there are $\frac{3}{4}$ colored and $\frac{1}{4}$ Himalayan. Himalayan albinism, c^h , and color are therefore alleles! It is interesting to see what happens when

Himalayan is crossed with albino. If Himalayan and albino are due to changes in different genes, each type should carry the allele of the other and reversion to full color should occur in F_1 . Actually the cross of Himalayan by albino produces *all* Himalayan in F_1 and $\frac{3}{4}$ Himalayan and $\frac{1}{4}$ albino in F_2 . Reversion does not occur. Other experiments show that the Himalayan allele and the albinism allele are never present in the same gamete; a colored animal may carry *either* Himalayan or albino but never both. It is evident that Himalayan and albino are allelic to each other and that both are allelic to full color. The Himalayan and albino alleles apparently arose by different mutations of the same gene.

Several other coat colors showing reduction in intensity of black and elimination of yellow also behave as alleles of albino and of Himalayan. These show the absence of dominance which is more usually found among the mutant alleles. For example, when chinchilla, which is less intense in color than the wild gray or agouti type, is crossed with albino, the F_1 animals are light gray intermediate between the parents and the F_2 consists of $\frac{1}{4}$ chinchilla, $\frac{1}{2}$ light gray, and $\frac{1}{4}$ albino. This shows that F_1 is heterozygous for an allele of albinism, chinchilla (c^{ch}), and for albinism (c^a). Such heterozygotes which contain unlike members of an allelic series are known as *compounds* to distinguish them from other heterozygotes. The genotypes of these members of the albino series in rabbits may therefore be written as follows:

Genotype	Phenotype (at normal temperature)
$C^+ C^+$	wild type
$C^+ c^{ch}$, $C^+ c^h$, $C^+ c^a$	wild type
$c^{ch} c^{ch}$	chinchilla
$c^{ch} c^h$, $c^{ch} c^a$	light gray
$c^h c^h$	Himalayan
$c^h c^a$	Himalayan
$c^a c^a$	albino

There are, in addition, several other alleles which are lighter than the wild type, darker than Himalayan. The wild-type allele is dominant to all the mutant alleles. Likewise, Himalayan is dominant to albino. Otherwise, the compounds are intermediate in coat color. All the mutant alleles and their compounds show the temperature sensitivity already described for Himalayan (p. 11).

Similar series of alleles of the albino gene are known in the mouse, the rat, the guinea pig, and the cat. In man it is possible that some persons with very little pigment in hair and skin also contain an allele of albinism. Human albinos with almost no pigment in skin, hair, or eyes (which are thus given a pink color by the blood which is visible through the transparent iris) are, like the full albinos of other mammals, homozygous for the

lowest member of this allelic series. The mutation from full color, *C*, to the albino allele, *c^a*, has occurred in nearly all mammals; but, because of the weakness of eyesight and sensitiveness of unpigmented skin, albinos are very seldom found in the wild state.

One series of alleles among the many known in *Drosophila melanogaster* has been extensively studied. This is the so-called "white-eye" series, since it consists of alleles of white, the first mutant which was found. The homozygotes form a series of eye colors of increasing intensity from white, through yellowish, to red, as shown in the partial list in Table IX. In this

TABLE IX.—ALLELES OF WHITE IN *Drosophila melanogaster*

Allele	Symbol	Allele	Symbol
white.....	<i>w</i>	apricot.....	<i>w^a</i>
ivory.....	<i>wⁱ</i>	cherry.....	<i>w^{ch}</i>
pearl.....	<i>w^p</i>	eosin.....	<i>w^e</i>
tinged.....	<i>w^t</i>	blood.....	<i>w^{bl}</i>
buff.....	<i>w^{bf}</i>	coral.....	<i>w^{co}</i>
honey.....	<i>w^h</i>	red (wild type).....	<i>W</i>

series, the compounds are in general intermediate between the parent homozygous types, while the wild-type allele is dominant to all others. Although the alleles of this gene seem to bear a quantitative relation to each other in respect to eye color, some of the alleles have other effects on testis color and spermatheca shape in which this quantitative variation is not apparent.

Many instances of multiple alleles are known among plants. In the snapdragon, Baur found a series of nine alleles all affecting the flower color (Fig. 33) and leading from deep red through various paler shades to ivory color with or without red stripes. Each allele appears to be more or less dominant to those lighter than itself.

Mosaic Dominance. Members of a series of multiple alleles may affect different combinations of traits or body parts of the organism. Stadler has described a series of alleles of the gene *R* in maize which determine the purple (anthocyanin) coloration of different parts of the plant. Four of these alleles have the following effects:

R^r—colored aleurone, colored plant.

R^e—colored aleurone, colorless plant.

r^r—colorless aleurone, colored plant.

r^e—colorless aleurone, colorless plant.

In compounds, the colored condition is dominant over the colorless. Thus, *R^rr^r* plants have both colored aleurone and colored leaves and stalks. The effects of this gene are, then, not on color in general but occur through

at least two components, one affecting aleurone color and the other plant color. Furthermore, it has been found that the aleurone color component may change (mutate) separately from the plant color component, although the two kinds of changes are not independent.

Different alleles of the gene *scute* in *Drosophila* remove different combinations of bristles on the fly's body. The compounds have all the bristles which are present in homozygotes for either allele and lack only those bristles which are removed by both alleles. An even more remarkable case of this sort has been described by Tan in the beetle *Harmonia axyridis*, in which different alleles produce black coloration of different parts of the

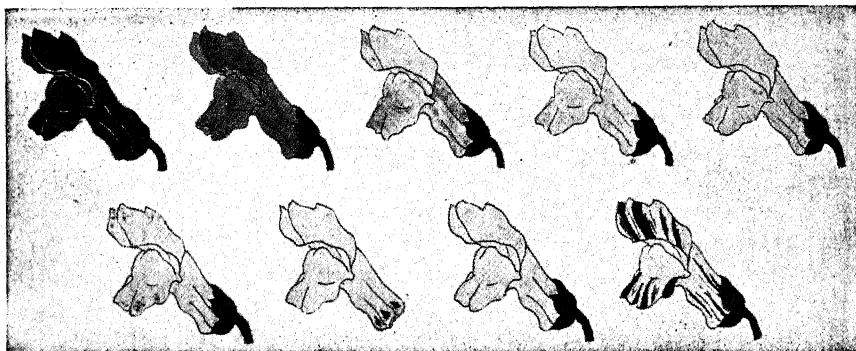


FIG. 33. A series of nine alleles of the gene *Pal* affecting flower color in the snapdragon (*Antirrhinum*), from normal red (upper left) through pale shades to red-striped (lower right). (After Baur.)

wing covers (elytra). The compounds have black coloration on any part of the wing covers which is made black by either allele separately. In the house mouse, one series of alleles shows both dominance and mosaicism (Table X).

TABLE X

Genotype	Phenotype
$A^Y A^L, A^Y A^+, A^Y a^t, A^Y a$	yellow
$A^L A^L, A^L A^+, A^L a^t, A^L a$	agouti back, light belly (yellow or white)
$A^+ A^+, A^+ a$	agouti back and belly
$A^+ A^t$	agouti back, light belly
$a^t a^t, a^t a$	black back, light belly
$a a$	black back, black belly

Blood Groups in Man. An interesting and important series of multiple alleles has been studied in man, and these too show the relationship that in a compound each allele produces its own effect. The character affected by these alleles is the property (antigen) of the normal red blood cells by which they respond to specific components in the blood serum (antibodies).

The antigen-antibody relationship is one of great specificity, like that between lock and key. Each antigen and its associated antibody has a peculiar chemical configuration, probably derived from its protein structure. Landsteiner discovered in 1900 that in certain cases, when the red blood cells of one person were placed in the blood serum of another, the cells were clumped, or agglutinated. If transfusions were made between two such incompatible persons, the introduced cells were likely to clump and occlude the capillaries in the recipient, resulting in shock and sometimes in death. This reaction occurred only when the cells of certain individuals were placed in serum from certain other persons. It was found that in respect to blood cells there are two such antigens, A and B, and two serum antibodies which agglutinate them. It was found that all persons could be classified into four groups with regard to the antigen property of the blood, those with antigen A (group A), those with antigen B (group B), those with both A and B (group AB) and those with neither antigen (group O) (Fig. 34). Persons of group A have no antibody which agglutinates A cells but do have antibodies which agglutinate B cells; those of group B have no antibodies which agglutinate B but do have antibodies which agglutinate A; those of group AB have neither type of antibody; those of group O have both types of antibody. When blood cells from persons of one group are placed in serum from persons of the same or another group, the reactions occur as shown in Table XI. These groups are now known as the "classical" blood groups to distinguish them from more recently discovered blood types.

TABLE XI

Blood group	Serum agglutinates blood cells of group	Cells agglutinated by serum of group
AB	None	O, A, B
A	B, AB	O, B
B	A, AB	O, A
O	A, B, AB	None

Large numbers of persons have been classified into these four groups by means of the agglutination test, and the distribution of the blood groups in the offspring of parents of known blood groups has been studied. The evidence shows that these blood properties are determined by a series of three allelic genes, I^A , I^B , and i , as follows:

Group	Genotype
AB	$I^A I^B$
B	$I^B I^B$ or $I^B i$
A	$I^A I^A$ or $I^A i$
O	ii

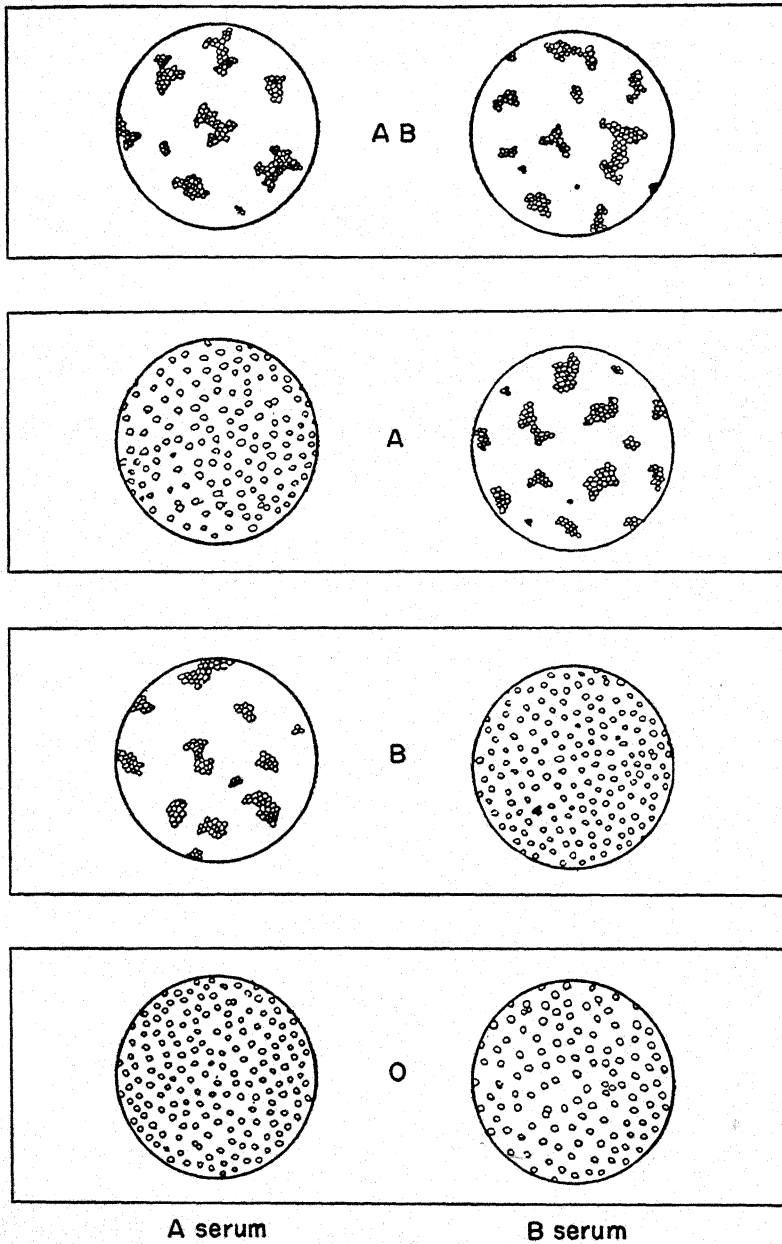


FIG. 34. Appearance of red blood cells of the four "classical" blood groups when tested by A serum (left) and B serum (right). (From Snyder, *Blood Grouping in relation to Clinical and Legal Medicine*, by permission of The Williams & Wilkins Company.)

I^A is a gene for the production of antigen (isoagglutinin) A, I^B for antigen B, and i for neither antigen. There are other alleles, I^{A_2} , I^{A_3} , etc., responsible for antigens which give weaker reactions than A, but these will be omitted for the sake of simplicity.

The existence of these alleles in man and the ease of recognizing the blood groups have obvious practical applications in blood transfusion, cases of disputed parentage, and description of human populations. What is significant for genetics is that allelic genes, affecting fundamental serological properties of the blood, act in such a way that in the compound $I^A I^B$ each allele produces its own characteristic and specific effect to the full so that the cells contain both antigens A and B; I^A and I^B , on the other hand, each show complete dominance over i , the lack of both antigens.

A new group of genes affecting the character of the antigens of human blood has been discovered recently through the work of Landsteiner, Wiener, Levine, and others. It was found that the red cells of about 85 per cent of the white population of New York were agglutinated by a serum prepared by immunizing rabbits against the blood of Rhesus monkeys. The antigen responsible was thus called the Rhesus, or Rh, factor. The people with this antigen, known as Rh-positives, proved to be either homozygous or heterozygous for a gene R (RR or Rr) which is responsible for the antigen, while Rh-negative people are rr . The study of this factor was stimulated by the discovery that infants suffering from an anemia known as erythroblastosis foetalis are usually Rh-positive, their mothers being Rh-negative, and their fathers Rh-positive. It was assumed that such infants inherit an R gene from the father, that the Rh antigen from the unborn child (usually from a succession of Rh-positive fetuses in previous pregnancies) causes the production of Rh antibodies in the mother, and that when these gain sufficient concentration in the mother's blood they attack the red cells of the fetus (which contain the Rh antigen) and hemolysis and erythroblastosis result (Fig. 35). It has now become apparent, however, that there are several Rh antigens and possibly even several genes responsible. Human populations of different countries differ in the relative frequencies of these alleles. Fisher has recently expressed the opinion that different antigens of the Rh series are produced not by multiple alleles of the same gene as in the classical blood groups but rather by three separate groups of alleles closely adjacent to each other in the same chromosome (see Chaps. IX and X). The problem needs further study.

Self-sterility, or -incompatibility Alleles. Most higher plants and many lower animals are, as we know, hermaphrodites which produce normally functioning gametes of both sexes. In some hermaphrodites such as the peas used by Mendel, self-fertilization occurs normally, and no barrier exists

to the union of the male and female gametes of the same individual. In other hermaphrodites such as the sea squirt *Ciona*, a peculiar self-sterility or self-incompatibility prevents the effective union of eggs and sperm of the same individual. Among the higher plants all the members of certain species are entirely self-sterile and will set seed only when pollinated by a different individual of the same species. With certain other individuals, however, these same plants are also cross-sterile.

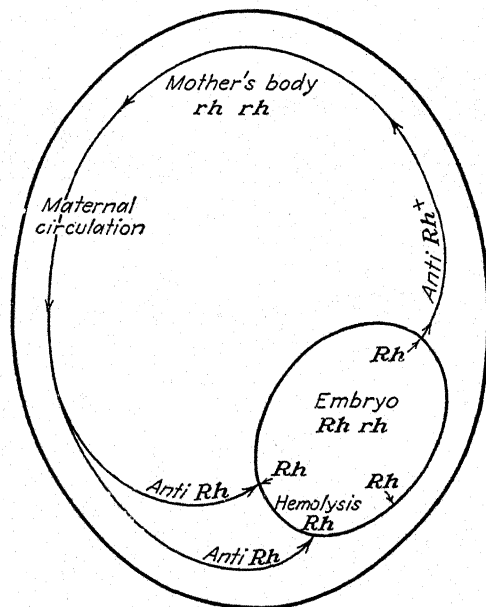


FIG. 35. The induction of anti-Rh antibodies in the mother's blood by Rh antigen in the embryo. The mother's genotype is rr ($rh\ rh$), the father's, RR ($Rh\ Rh$).

East and his coworkers discovered that in the genus *Nicotiana* (tobacco) these self- and cross incompatibilities are due to the existence of a long series of alleles of one gene, designated as $S^1, S^2, S^3, \dots, S^n$. Pollen grains containing a certain allele, say S^1 , fail to grow properly in the style of a plant which carries the same allele. Thus, if a plant S^1S^2 is pollinated by its own pollen or by the pollen of another S^1S^2 plant, no pollen grains will reach the ovules in time to effect fertilization. But a cross of S^1S^2 ♀ by S^2S^3 ♂ yields offspring of classes S^1S^3 and S^2S^3 only. S^2S^3 ♀ \times S^1S^2 ♂ gives offspring S^1S^3 and S^1S^2 . In all crosses in which the parents have one allele in common, the mother's class is not found among the offspring, which means that the presence of an allele in the mother's tissue prevents the growth of pollen grains which carry that same allele. If, however, a plant

S^1S^2 is crossed with S^3S^4 , each of the reciprocal crosses yields offspring of all four types, S^1S^3 , S^1S^4 , S^2S^3 , and S^2S^4 . This hypothesis, which is illustrated in Table XII, has been tested in various ways and gives a satisfactory explanation of the results not only in *Nicotiana*, where 15 alleles have been found, but in several other genera as well. In *Oenothera organensis*, Sterling Emerson found 37 different compatibility alleles in 500 plants. Even longer series have been detected in red clover.

TABLE XII.—PROGENY OBTAINED FROM THREE INCOMPATIBILITY CLASSES IN NICOTIANA

		Male Parent		
Female Parent		$S^1 S^3$	$S^1 S^2$	$S^2 S^3$
	$S^1 S^3$	No offspring	$S^1 S^2$ $S^2 S^3$	$S^1 S^3$ $S^2 S^3$
	$S^1 S^2$	$S^1 S^3$ $S^2 S^3$	No offspring	$S^1 S^3$ $S^2 S^3$
	$S^2 S^3$	$S^1 S^2$ $S^1 S^3$	$S^1 S^2$ $S^1 S^3$	No offspring

The alleles determining self-sterility apparently produce their effect by controlling the rate of pollen-tube growth. In compatible combinations the tubes grow more and more rapidly as they approach the ovule, but in incompatible ones they grow so slowly that before the gamete reaches the ovule the flower has withered. This is probably due to a gene-controlled reaction between the diploid tissue of the female sporophyte and the haploid gametophytic tissue of the pollen tube. In this reaction each allele acts separately. Thus, a plant S^1S^3 discriminates against both S^1 and S^3 pollen by opposing the growth of S^1 and S^3 pollen tubes, whence such alleles have been called *oppositional* alleles. The separate action of such alleles resembles that of I^a and I^b in the blood group AB, in which two separate antigens are produced without interference. This mosaic type of effect is quite different from the interaction between alleles in cases of incomplete dominance and has led to the suggestion that alleles which in compounds act independently of each other perhaps give rise to substances such as antigens.

Pseudoalleles. A few cases have been studied in which several genes with related effects like those common to members of an allelic series have turned out not to be alleles but due to alterations in separate genes which are so close together that in the great majority of gametes they behave as alleles. Since the resolution of such cases requires an understanding of the spatial relations of genes in chromosomes, they are discussed later in

Chapter X. They do not affect the validity of one of the basic assumptions of genetics, that genes exist in two or more alternative states known as alleles which, with each other, show segregation but not recombination.

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PROBLEMS

Note. In certain breeds of sheep both sexes bear horns, but in others horns are absent from both. In crosses between the two, the horned condition is dominant in males and recessive in females. White fleece is dominant over black in both sexes and in all breeds.

102. If a homozygous horned, white ram is bred to a homozygous hornless, black ewe, what will be the appearance of the F_1 and the F_2 generations as to horns and color?

103. A horned, black ram bred to a hornless, white ewe has the following offspring: Of the males, one-fourth are horned, white; one-fourth horned, black; one-fourth hornless, white; and one-fourth hornless, black. Of the females, one-half are hornless, black and one-half hornless, white. What are the genotypes of the parents?

Note. In man, assume that baldness, *S*, is dominant over nonbaldness, *s*, in males but recessive in females.

104. A brown-eyed, bald man whose father was nonbald and blue-eyed marries a blue-eyed, nonbald woman whose father was bald and all of whose brothers were

also bald. What will be the probable appearance of their children as to eye color and baldness?

105. In rabbits full color (C), Himalayan albinism (c^h), and albinism (c^a) form a series of multiple alleles with dominance in the order given. What will be the appearance of the offspring of the following crosses:

- (a) colored \times Himalayan (homozygous)
- (b) F_2 from (a)
- (c) Himalayan \times albino
- (d) F_2 from (c)
- (e) F_1 from (a) \times F_1 from (c)

106. What are the genotypes of the parents in the following crosses in rabbits:

- (a) wild type \times wild type, giving $\frac{3}{4}$ wild type: $\frac{1}{4}$ Himalayan
- (b) wild type \times wild type, giving $\frac{3}{4}$ wild type: $\frac{1}{4}$ light gray
- (c) wild type \times Himalayan, giving $\frac{1}{2}$ wild type: $\frac{1}{4}$ Himalayan: $\frac{1}{4}$ albino

107. What will be the appearance of the offspring from the following crosses in rabbits (c^hc^h is chinchilla; c^hc^h and c^hc^a are light gray):

- (a) $Cc^h \times Cc^a$
- (b) $c^hc^h \times c^hc^h$
- (c) $c^hc^a \times c^hc^h$
- (d) $Cc^h \times c^hc^a$

Note. In mice the following genes affecting coat color form a series of multiple alleles: A^y , yellow; A^L , agouti light belly; A , agouti (gray belly, wild type); a^t , black and tan (black back, light belly); a (black, nonagouti); Aa^t animals are agouti with light belly; otherwise dominance is complete in order given. C is colored; cc , albino.

108. What will be the appearance of the offspring of the following crosses:

- (a) $A^LA \times A^LA$
- (b) $A^LA^L \times A^La$
- (c) $A^La^t \times A^La$
- (d) $A^LA \times a^ta$
- (e) $A^yA \times A^yA^L$
- (f) $A^ya \times A^ya^t$
- (g) $A^ya^t \times A^ya^t$

109. What are the genotypes of the parents in the following crosses:

- (a) Agouti light belly \times agouti light belly, giving one-fourth agouti, one-half agouti light belly, one-fourth black and tan.
- (b) Agouti light belly \times agouti, giving one-half agouti light belly; one-fourth agouti, one-fourth black.
- (c) Albino \times agouti, giving all agouti light belly in F_1 ; and in F_2 three-sixteenths agouti, three-eighths agouti light belly, three-sixteenths black and tan, and one-fourth albino.

110. In maize there are several factors which affect plant color. Three of these are *sun red*, *weak sun red*, and *dilute sun red*, their intensities being in the order named. Following are the results of crosses between these three types (data from Emerson): sun red \times dilute sun red gave all sun red in F_1 and 998 sun red: 314 dilute sun red in F_2 ; weak sun red \times dilute sun red gave all weak sun red in F_1 and

1,300 weak sun red: 429 dilute sun red in F_2 ; sun red \times weak sun red gave all sun red in F_1 and 71 sun red:16 weak sun red in F_2 .

Explain these results, stating the relationship among these three plant colors.

111. What will be the phenotype, as to blood groups, of offspring of parents of the following genotypes for blood groups:

$$I^A i \times I^B i$$

$$I^A I^B \times I^B i$$

$$I^B i \times I^B i$$

112. If a person of blood group AB marries one belonging to group O, what will be the blood groups of their children?

Note. In the three following problems on blood groups, determine the genotypes of the parents.

113. One parent is group A and the other group B, but all four groups are represented among the children.

114. Both parents are group A, but three-fourths of the children belong to group A and one-fourth to group O.

115. One parent is AB and the other B, but of the children one-fourth are A, one-fourth AB, and one-half B.

116. In the two following cases of disputed paternity, determine the true father of the child:

(a) The mother belongs to group B, the child to O, one possible father to A and the other to AB.

(b) The mother belongs to group B, the child to AB, one possible father to A and the other to B.

117. In the choice of donors for blood transfusion, a patient's brother or sister is often selected. Would these be more likely to be successful donors if both parents belonged to blood group AB or if both belonged to group O? Explain.

118. In *Nicotiana*, if s^1s^2 is crossed with s^4s^5 , calculate the proportion of cross-sterile (incompatible) combinations amongst the progeny.

119. If the progeny plants from Table XII are allowed to self-fertilize or to cross at random in large numbers, what should be the distribution of genotypes in the next generation, assuming that all genotypes are equally viable?

120. In *Nicotiana*, 100 plants when tested among themselves for self- and cross-sterility were found to fall into groups which had the following relationships in respect to offspring:

		Female Parent			
		A	B	C	D
Male Parent	A	—	$\frac{1}{2}$ A, $\frac{1}{2}$ C	$\frac{1}{2}$ A, $\frac{1}{2}$ B	$\left\{ \begin{array}{l} \frac{1}{4} \text{ B, } \frac{1}{4} \text{ C} \\ \frac{1}{4} \text{ E, } \frac{1}{4} \text{ F} \end{array} \right.$
	B	$\frac{1}{2}$ B, $\frac{1}{2}$ C	—	$\frac{1}{2}$ A, $\frac{1}{2}$ B	$\frac{1}{2}$ B, $\frac{1}{2}$ E
	C	$\frac{1}{2}$ B, $\frac{1}{2}$ C	$\frac{1}{2}$ A, $\frac{1}{2}$ C	—	$\frac{1}{2}$ C, $\frac{1}{2}$ F
	D	$\left\{ \begin{array}{l} \frac{1}{4} \text{ B, } \frac{1}{4} \text{ C} \\ \frac{1}{4} \text{ E, } \frac{1}{4} \text{ F} \end{array} \right.$	$\frac{1}{2}$ D, $\frac{1}{2}$ E	$\frac{1}{2}$ D, $\frac{1}{2}$ F	—

Give the allelic constitutions of A, B, C, and D which would explain these relationships.

121. In about 85 per cent of Europeans known as *secretors*, antigens A and B are found in saliva and other body fluids as well as in the blood, while 15 per cent do not have the antigens in saliva and are known as *nonsecretors*. If *S* (secretor) is dominant to *s* (nonsecretor) and *S* and *s* are inherited independently of the classical blood-group alleles, calculate the proportion of offspring from the following matings which would be expected to give A or B reactions in the saliva:

AB secretor (*Ss*) \times O (*Ss*)

O nonsecretor \times AB secretor (*Ss*)

122. In a Chinese population, 99 per cent of all persons tested were Rh-positive, that is, had one of the Rh alleles. If matings in this population are at random, the frequency of gene *r* should be $\sqrt{1}$ per cent, or 10 per cent, and of *R* = $100 - \sqrt{1}$ per cent, or 90 per cent. What proportion of the Rh-positive men in this population would you expect to be *RR*?

123. In the population above, what proportion of all marriages would be such that an erythroblastotic baby might be produced?

124. Assuming that the three main AB blood-group alleles *I^A*, *I^B*, and *i* are inherited independently of the six Rh alleles (*R¹*, *R²*, *R³*, *R⁴*, *R⁵*, *r*) assumed by certain investigators, how many genotypes with respect to these two blood characters are theoretically possible?

CHAPTER V

THE EXPRESSION AND INTERACTION OF GENES

Although the laws of segregation and of independent assortment were immediately confirmed following the "rediscovery" of Mendel's work in 1900, they appeared to apply only to certain kinds of hybrids like those studied by Mendel. Hereditary transmission in other crosses, as in those between species, or between individuals differing in quantitatively varying characters, often appeared to follow other rules, referred to as "blending inheritance" or non-Mendelian inheritance or complex inheritance. Indeed, the classical Mendelian ratios, such as $\frac{3}{4}:\frac{1}{4}$ and 9:3:3:1, which are due to full dominance, independent assortment, and lack of interference between the effects of different factors, do not occur in all crosses. It was soon found, however, that many of the apparent exceptions could be explained by assuming that certain characters depend for their expression on the interaction of two or more pairs of genes. Depending upon the form the interaction takes in particular cases, the ratios were found to be modified in various ways, while the fundamental laws of transmission remained the same. Thus the inheritance of a very wide range of traits in many kinds of animals and plants was shown to follow Mendel's laws and could be analyzed in terms of genes. As exception after exception fell into line, it became increasingly evident that with the exception of some traits inherited through the cytoplasm (see Chap. XVII) the transmission of genes accounts for all biological heredity. Some of the more important forms of gene expression and interaction, resulting in departures from Mendel's original ratios, will be discussed in this and the following chapter.

Ratios Modified by Gene Expression. In general we can recognize gene differences and follow them in inheritance only by means of their phenotypic effects. The ratios in which different phenotypes appear in the progeny disclose at once the genotypes of the parents and some information about the phenotypic effect of the genes involved. Thus from a 3:1 ratio we conclude that, under the conditions given, one allele is dominant to the other and from a 1:2:1 ratio that each allele has equal dominance. When phenotypic ratios other than those due to such differences in dominance are obtained, our first question is whether something has upset the fundamental mechanism of segregation and recombination or whether peculiarities in the phenotypic expression and interaction of the genes are

responsible. In the following sections a number of modifications of Mendelian ratios due to the latter causes are discussed and shown not to involve any exceptions to the principles of segregation or independent assortment.

Lethal Genes (the 2:1 Ratio). Not long after Mendel's work was re-discovered, it was noticed by Cuénot that the yellow variety of the house mouse never breeds true, and he concluded that all yellow mice are hybrids. Yet matings between two yellow mice were later found always to produce

TABLE XIII

Parents	Yellow	Nonyellow
Yellow \times nonyellow.....	2,378	2,398
Yellow \times yellow.....	2,396	1,235

progeny in a ratio of about 2 yellow:1 nonyellow rather than in the 3:1 ratio expected. The results of a number of investigators added together are shown in Table XIII. The progeny of the cross yellow \times nonyellow segregates in a 1:1 ratio, indicating that all of the yellows were heterozygous; the 3:1 expectation for yellow \times yellow does not fit the data, while the 2:1 expectation fits the data very well. In addition it was found that

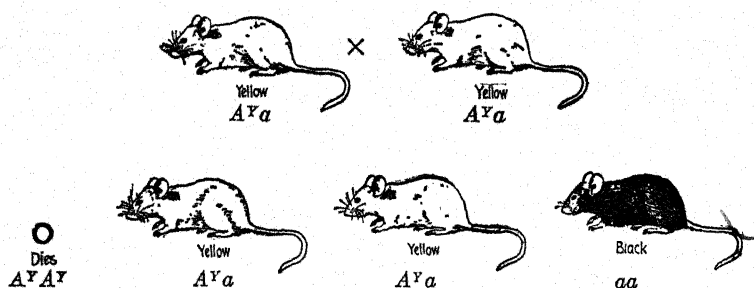


FIG. 36. Inheritance of a lethal factor in mice. A mating of yellow by yellow giving $\frac{1}{4}$ dead embryos, $\frac{1}{2}$ yellow mice, and $\frac{1}{4}$ nonyellow ones.

the litters born from matings between yellows are smaller by about one-fourth than litters from yellow \times nonyellow. Castle and Little showed that all these facts could be reconciled by assuming that of the three classes of zygotes expected from yellow by yellow matings (one-fourth homozygous yellow, two-fourths hybrid yellow, one-fourth nonyellow) only the latter two were born in the proportion 2 yellow:1 nonyellow. The homozygous

yellow class was assumed to be inviable; that is, two "doses" of yellow had a lethal effect on the embryo. This was confirmed by several later investigators who found that one class of embryos from yellow by yellow matings regularly died in an early embryonic stage. (Fig. 36.)

The inheritance of yellow may be represented as follows:

	A^Y = a dominant factor for yellow			
	a = a recessive factor for nonyellow			
Parents.....	Yellow $A^Y a$	×	Yellow $A^Y a$	
Gametes.....	A^Y, a		A^Y, a	
Progeny.....	$A^Y A^Y$	$A^Y a$	$A^Y a$	aa
	$\frac{1}{4}$ Lethal die		$\frac{1}{2}$ yellow live	$\frac{1}{4}$ nonyellow live

Thus, a mouse which inherits the yellow lethal can survive only if it receives also the viable allele for nonyellow. The reaction caused by two yellow alleles which is fatal to the developing embryo has not yet been identified. These and other questions of interest in connection with the large class of genes with lethal or sublethal effects will be discussed later (Chapter XVI). At present it should be pointed out that "normal" ratios are obtained only if the different genotypic classes have about equal viabilities. A gene like yellow, with a dominant effect on coat color and a recessive lethal effect, will regularly give ratios of 2:1 among the viable zygotes. A case of partial lethal effect in rats, in which one class of zygotes is not absent but falls below expectation was pointed out on page 50.

In *Drosophila* and many other experimental animals and plants it has been noticed that many mutant genes reduce the viability; and the F_2 populations in which such genes are segregating often contain too few of the mutant type.

In man, several lethal genes have been detected. One, juvenile amaurotic idiocy, results in the death of homozygous children before the age of eighteen; infantile amaurotic idiocy, or Tay Sachs disease, which results in death in the first few years, is due to another recessive gene. It has been found that the parents of children dying of Cooley's anemia, also called Mediterranean anemia, or thalassemia major, always show a mild anemia and minor abnormalities of the red blood cells known as thalassemia minor. Thalassemia minor appears to be the heterozygous expression of the gene which when homozygous causes thalassemia major. Progenies from heterozygotes, when examined as young children, should therefore show $\frac{1}{4}$ with normal blood picture, $\frac{1}{2}$ with thalassemia minor, and $\frac{1}{4}$ with thalassemia major. Since the latter die before puberty, progenies examined after the children have grown up should show $\frac{2}{3}$ with thalassemia minor and $\frac{1}{3}$ normal, just as in the case of the yellow mouse.

It is quite likely that lethal and sublethal effects are not always necessary consequences of the action of a single gene since the lethal effect may sometimes be reduced or "cured" by changing its environment or other factors in the genotype. Thus homozygous yellow embryos live a little longer in the uterus of a nonyellow mother (into which a "yellow" ovary has been grafted) than in a yellow mother. Similarly, albino mutations in plants generally act as lethals since they interfere with chlorophyll production; but in some cases they may be kept alive by grafting on normal plants. Like dominance, the lethal effect is a characteristic of a particular genotype under a particular set of conditions.

Ratios Modified by Interaction of Different Genes. Combs in Fowls. The first case of this kind was discovered a number of years ago by Bateson and Punnett, during the course of experiments on the inheritance of comb form in fowls. Each of the common varieties of poultry possess a characteristic type of comb as shown in Fig. 5 (p. 12). The Wyandotte breed, among others, has a low, regular, papillate comb known as the "rose" comb; Brahmas and some of the varieties of game fowls have a narrower, higher, three-ridged comb known as the "pea" comb; Leghorns and breeds of similar origin have "single" combs, consisting of a single upright blade. Each of these types can be bred true (Fig. 37). Crosses made between rose-combed and single-combed varieties showed that rose was dominant over single and that there was a segregation into three-fourths rose and one-fourth single in the F_2 . In crosses between pea-combed and single-combed birds, pea comb was also found to be dominant over single, and a simple 3:1 ratio appeared in the F_2 . A new and interesting result, however, was obtained when rose was crossed with pea, for the F_1 birds showed a new comb form different from either the rose or the pea. This was known as "walnut" comb from its resemblance to half of a walnut meat and had previously been noted as characteristic of the Malay breeds of fowls, a race unrelated to the types from which the new walnut comb was obtained. When the F_1 walnut-combed birds were bred together, a still more remarkable result was manifest, for in the F_2 generation there appeared not only walnut-, rose-, and pea-combed fowls but *single-combed* ones as well. After large numbers of F_2 birds had been bred and classified it was found that these types occurred in the proportions $\frac{9}{16}$ walnut, $\frac{3}{16}$ rose, $\frac{3}{16}$ pea, $\frac{1}{16}$ single.

This was recognized as the ratio to be expected in F_2 from a cross of parents differing in *two* genes. The doubly dominant class in F_2 was apparently walnut, while the numbers of singles obtained indicated that this type contained both of the recessive genes involved, a conclusion supported by the fact that the F_2 singles when bred together produced only single-

combed progeny in subsequent generations. The following explanation of these results was offered: The walnut comb depends on the presence of *two* dominant genes, *R* and *P*. One of these genes alone (*R*) produces the rose comb; the other alone (*P*) produces the pea comb. The combination of

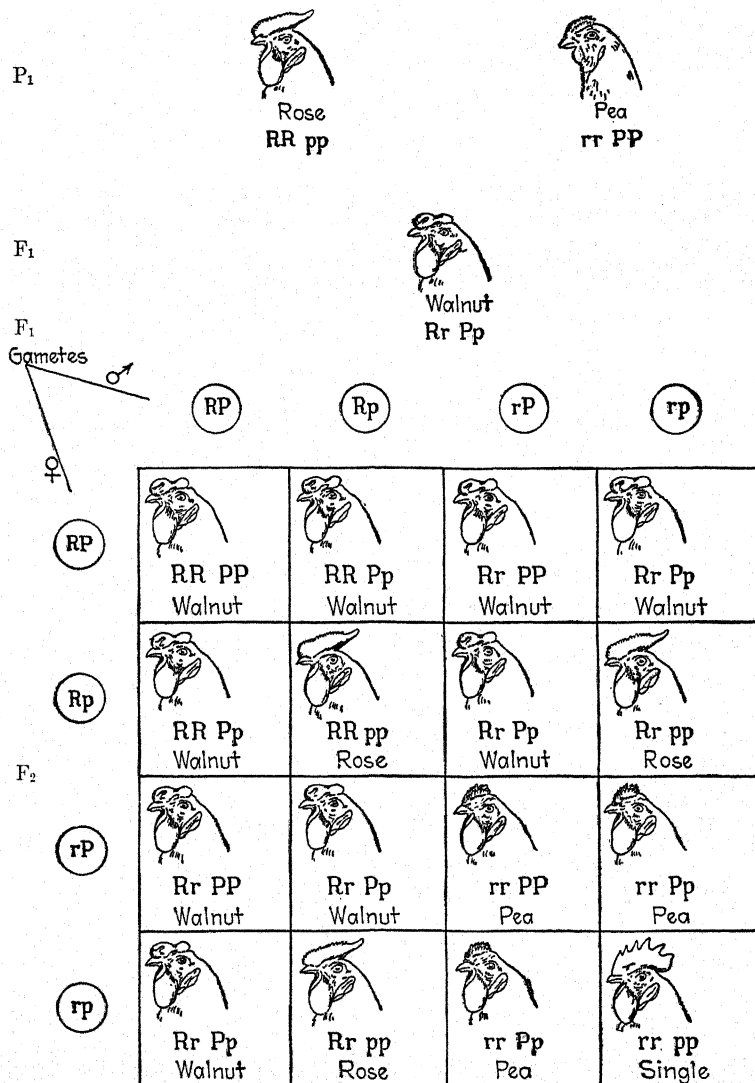


FIG. 37. Diagram showing interaction of genes for comb form in fowls. The cross of a pure rose-comb bird with a pure pea-comb one gives all walnut-combed offspring. The 16 possible combinations of the F_1 gametes, with their genotypes and the phenotypes resulting from gene interaction, are shown in the F_2 checkerboard.

the recessive alleles of these genes produces the single type of comb *rp*. These assumptions are illustrated in the diagram in Fig. 37.¹

The similarity between the F_2 results in this diagram and the common two-gene case explained on page 62 will be readily noticed. The mode of inheritance of the genes for rose and pea does not differ at all from the usual Mendelian scheme. The differences which distinguish this and similar cases from ordinary dihybrid inheritance are that (1) the F_1 resembles neither parent and (2) *two new types* appear in F_2 . One of these new characters (walnut comb), therefore, evidently results from an interaction between two independently inherited dominant genes, while the other (single comb) results from the interaction of their two recessive alleles. These peculiarities are due not to a new method of inheritance but simply to the circumstance that both genes involved happen to express themselves in the same part of the organism, in this case the comb.

Fruit Shape in Squashes. In the summer squash, *Cucurbita pepo*, races breeding true to different fruit shapes have been isolated. The spherical form behaves as a recessive to the flat or disk form (Fig. 26, p. 61). A cross of two spherical-fruited races from different ancestry, however, produced in F_1 only *disk*-fruited offspring, and in F_2 *three* types of plants appeared: nine-sixteenths with disk fruits, six-sixteenths with spherical fruits, and one-sixteenth with *elongate* fruits. This ratio shows that two pairs of genes are involved. Interaction between the two dominant alleles gives rise to disk and between the two recessives produces the new combination *elongate*, while either gene alone results in the sphere shape. Thus:

P ₁	Sphere × Sphere			
	AAbb		aaBB	
F ₁	Disk			
	AaBb			
F ₂	$\frac{9}{16}$ Disk	$\frac{3}{16}$ Sphere	$\frac{3}{16}$ Sphere	$\frac{1}{16}$ Elongate
	A(a)B(b) ²	A(a)bb	aaB(b)	aabb

The peculiarities here are (1) the resemblance of F_1 to neither parent, (2) the appearance of *one* new type in F_2 , and (3) the phenotypic resemblance of two different genotypes in F_2 . As in the case of comb form, the genes affect the same character, which is thus influenced by an interaction between two pairs of independent genes.

¹ The checkerboard scheme in this diagram, which is similar to that employed in the study of a simple dihybrid, is a useful means of finding the various genotypes resulting from combinations of the gametes formed by complex heterozygotes of this sort. The relative proportions of the different types of individuals to be expected may be read off directly.

² Factor symbols enclosed in parentheses represent alternative genotypes. Thus $A(a)$ indicates that the genotype may be either AA or Aa .

Flower Color in Sweet Peas (Complementary Genes). A similar type of interaction has been found between two factors affecting flower color in the sweet pea, *Lathyrus odoratus*. This plant occurs in a number of true-breeding varieties, all descended from the wild sweet pea of Sicily, which bears a purple flower with red wings. While studying a number of the different cultivated varieties, Bateson and Punnett found that purple flower color is dominant over white and gives a typical 3:1 ratio in the F_2 . They also observed that the white types bred true, as was to be expected, and that crosses between white varieties usually produced white-flowered progeny. In one instance, however, where two pure white varieties were crossed, there resulted quite unexpectedly no white offspring at all but only *colored-flowered* plants. The flowers of these F_1 hybrids were very similar in color to the wild Sicilian ancestor of the cultivated sweet pea. When such purple-flowered F_1 plants were self-fertilized, they produced an F_2 generation consisting of about nine-sixteenths purple-flowered plants and seven-sixteenths white-flowered ones. All the F_2 white individuals bred true when self-fertilized. The purples, however, were evidently of several different types, for a few bred true; others produced colored- and white-flowered plants in the proportion of three-fourths colored to one-fourth white, while still others produced offspring of which about nine-sixteenths were colored and seven-sixteenths white.

This result, like the inheritance of comb shape, may also be explained by segregation of two independent pairs of genes, but the type of interaction between them is somewhat different, for no new traits appear in the F_1 or F_2 . The fact that purple flower color occurs in nine-sixteenths of the F_2 plants suggests that it appears only when two independent dominant genes are present together and that it results from some sort of interaction between them. White flower color may thus evidently be due to the absence of either or both of these genes. Denoting one of the genes by C (color) and the other by P (purple), it may be assumed that one white parent was of the genotype $CC\ pp$ while the other was $cc\ PP$. Neither C alone nor P alone is, by this assumption, able to cause the production of color in the flowers. The cross between two such white types produces the heterozygote $Cc\ Pp$, which bears colored flowers, since it contains both the genes for color and purple. When this hybrid forms its gametes, C and P segregate independently, and the gametes formed may be written CP , Cp , cP , and cp . The combinations between these types of gametes and the resulting flower colors of the F_2 plants are shown in Fig. 38.

The ratio here is obviously the normal 9:3:3:1 expected in F_2 when the parents differ in two genes, but with the last three terms added together (9:7). The peculiarity of this ratio arises from the fact that *all* plants which lack either C or P are white, regardless of the condition as to the

other factor. Thus three F_2 plants are white because they lack C , three are white because they lack P ; while one white lacks both of these.

A study of the genotypes of the plants with colored flowers also explains why they breed so differently in later generations. One of them (with the genotype $CC PP$) breeds true to purple. Four (with the genotypes $Cc PP$ or $CC Pp$) produce about three-fourths purple and one-fourth white offspring when inbred, since they are homozygous for one of the factors.

P_1	White CC pp		White cc PP		
F_1	Purple Cc Pp				
	CP	Cp	cP	cp	
F_2	CP	CC PP Purple	CC Pp Purple	Cc PP Purple	Cc Pp Purple
	Cp	CC Pp Purple	CC pp White	Cc Pp Purple	Cc pp White
	cP	Cc PP Purple	Cc Pp Purple	cc PP White	cc Pp White
	cp	Cc Pp Purple	Cc pp White	cc Pp White	cc pp White

FIG. 38. The 9:7 ratio. Checkerboard showing the expected composition of the F_2 from a cross of two white-flowered sweet peas which produce all purple-flowered plants in F_1 .

Four others (with the genotype $Cc Pp$) produce nine-sixteenths purple and seven-sixteenths white, just as did the F_1 .

Cases of interaction such as this, in which two genes are similar in their individual effects but are both necessary to the production of another and different character, may be called *complementary*. Sometimes the genes participating in such interactions are called complementary genes, but this loose usage should not blind us to the fact that the particular effect of a gene depends not on itself alone but on the rest of the genotype in which it appears.

The interaction of two genes such as *C* and *P* to produce a character different from that which results from either one alone may be made clearer by a simple chemical comparison. When a colorless solution of an alkali (such as potassium hydroxide) and a colorless solution of an "indicator" (such as phenolphthalein) are brought together, a light red color appears. Here the chemical interaction of two colorless substances results in the production of color. The alkali may be compared to the material, whatever it is, which is furnished by the gene *C*; and the indicator, to that furnished by the gene *P*.

This illustration may be more than a mere analogy, for Blakeslee found that in the yellow daisy, *Rudbeckia hirta*, the cross of two yellow-coned races produces a purple-coned F_1 and the ratio of $\frac{9}{16}$ purple-: $\frac{7}{16}$ yellow-coned in F_2 . When placed in dilute alkali, the cones of one of the parental yellow races turn reddish; those of the other race, blackish. The yellow-coned types in F_2 , although identical in appearance, could be similarly differentiated by treatment with alkali into three-sixteenths of one type and four-sixteenths of the other. Evidently yellow results from chemically different processes in the two types; the combination of the two carries the reaction to a further stage (purple) than is possible with either factor alone, and this reaction may be simulated by the addition of alkali instead of the addition of another gene.

In sweet peas and several other plants it is now known that two white strains which on crossing give colored progeny each contains a *different* necessary component of the anthocyanin pigment. The combination of these components gives color, whether they be combined by crossing or as extracts in vitro. It is thus reasonable to suppose that in sweet peas the gene *C* leads to one of these components and *P* the other. The identification of a chemical substance with an immediate effect of a gene has been made in a few cases (*cf.* Chap. XVI).

Reversion. The method of inheritance of flower color in sweet peas suggests an explanation for the numerous instances among domesticated animals and plants in which crosses between true-breeding varieties produce progeny resembling a remote ancestor more than they do either parent. Plant and animal breeders had noted these peculiar "throwbacks," or "reversions," but in the absence of any satisfactory explanation they regarded reversion as the expression of some mysterious force which caused the retention and subsequent reappearance of a remote ancestral trait, a phenomenon to which the term atavism was applied. It is now known that such reversion may be explained in terms of ordinary Mendelian inheritance, for the reappearance of an old trait is usually due to the reunion of the two or more genes, necessary for its production, which had become separated in the history of the species. Thus, in the sweet pea, it is plain

from the experiment cited above that purple flower color depends on at least two genes and that white flower color results when either is changed. It is easy to imagine that one white variety arose when in the purple type a mutation occurred from C to c , while the second white variety arose when P changed to p . Thus the two elements necessary for purple color became separated into two different strains. When these strains were crossed, the two genes were reunited, and the primitive, or "reversionary," flower color appeared.

Coat Color in Rodents (the 9:3:4 Ratio). A similar but more complex case of gene interaction and reversion has been worked out in breeding experiments with "fancy" varieties of the common house mouse, where not only two but a number of genes have been found to interact in producing what appears to be a simple character. The ancestral, or original, coat color of this species is seen in the grayish-brown or grizzled pattern of our ordinary wild mice. When closely examined, this is found to be due to the presence of two pigments in the fur. The individual hairs are for the most part black with a narrow yellow band near the tip. The underside of the animal is usually much lighter, the hairs being cream or yellow, with some black or gray at the base. This inconspicuous and hence protective coloration, which is known as the "agouti" pattern, characterizes nearly all of the wild rodents, such as the Norway rat, the wild rabbit, the guinea pig, the gray squirrel, and many others.

A number of variations which have taken place in this wild gray or agouti coat coloration have been preserved under domestication and have given rise to the many color varieties of mice known to fanciers. The commonest and most familiar variation is the albino, in which the coat is white and the eyes are pink or blood color because of the entire absence of pigment from the iris. Albinos always breed true, and this variation has been found to behave as a simple recessive to any color. Another variation in coat color probably arose through the disappearance of all yellow pigment from the agouti pattern, leaving the fur solid black. Black is recessive to the wild gray type and breeds true. When black mice are crossed with ordinary albinos, the progeny are usually *all agouti* like the wild type. When these F_1 agoutis are inbred, their progeny consist, on the average, of nine-sixteenths agouti animals, three-sixteenths black, and four-sixteenths albino (Fig. 39). This, like the 9:7 ratio encountered in sweet peas, indicates a difference of two genes. Here, however, the last two terms of the ordinary 9:3:3:1 ratio have been added together, indicating that two of the ordinarily different classes of the dihybrid F_2 zygotes cannot be distinguished. The results are explained on the assumption that the parents differ in (1) a gene, C , necessary for the development of any color, which the black mice contain but which is lacking in the albinos; and in (2) a gene for the agouti

pattern, *A*, which results in a banding of the black hairs with yellow. Since the black mice cannot contain this gene *A* (or they would appear agouti), it must have come from the albino parent, where, in the absence of the ability to develop any color at all, it could have no visible expression. The recombination of these two genes, one for color and the other for the agouti pattern, reconstitutes the genotype of the wild mouse, and a "reversionary" type results.

P₁	Black CC aa		Albino cc AA		
F₁	Agouti Cc Aa				
	CA	Ca	cA	ca	
F₂	CA	CC AA Agouti	CC Aa Agouti	Cc AA Agouti	Cc Aa Agouti
	Ca	CC Aa Agouti	CC aa Black	Cc Aa Agouti	Cc aa Black
	cA	Cc AA Agouti	Cc Aa Agouti	cc AA Albino	cc Aa Albino
	ca	Cc Aa Agouti	Cc aa Black	cc Aa Albino	cc aa Albino

FIG. 39. The 9:3:4 ratio. Checkerboard showing the expected composition of the F₂ from a cross of black and albino mice which produce all agouti animals (wild type) in F₁.

This case is, therefore, similar to that of flower color in sweet peas in that (1) two independently inherited genes, both affecting the same part (in this case coat color), interact to produce a single character; and (2) this interaction produces reversion in the F₁ generation, followed by the reappearance in the F₂ of both of the parental colors as well as the reversionary type. It differs somewhat from the previous case, for in sweet peas *three* of the F₂ genotypic classes have the same appearance, producing a 9:7 ratio, whereas in mice only *two* of the F₂ genotypic classes are indistinguishable, thus producing a 9:3:4 ratio.

Epistasis. One of the first complications which was encountered in the discussion of heredity was the fact of dominance, by which the presence of one allele of a pair was obscured or hidden. It sometimes happens that when two *different* genes, which are not alleles, both affect the same part or trait of the organism, the expression of one covers up or hides the expression of the other. A gene which thus masks or prevents the expression of another is said to be *epistatic* to it, and the gene which is hidden is said to be *hypostatic*. This masking effect is known as *epistasis* and is similar to dominance except that it occurs between different pairs of genes instead of between the two members of an allelic pair.

In Squashes (the 12:3:1 Ratio). In summer squashes there are three common fruit colors, white, yellow, and green. In crosses between white and yellow and between white and green, white is always found to be dominant; and in crosses between yellow and green, yellow is always found to be dominant. Yellow thus acts as a recessive in relation to white but as a dominant in relation to green. There is evidently a gene, W , which is epistatic to those for yellow and green; and so long as it is present, no color is produced in the fruit, regardless of whether or not genes for color are present. Where this gene for white is lacking, however (in plants which are ww), the fruit color will be yellow if gene Y is present and green if it is absent. Green-fruited plants may thus be represented by the double recessive genotype $ww\ yy$, yellow-fruited plants by $ww\ YY$, and white-fruited ones either by $WW\ YY$ or by $WW\ yy$.

The truth of this assumption that there are two independent gene pairs, one epistatic over the other, may be tested by crossing a homozygous white from a race which is known to carry yellow, $WW\ YY$, with a green, $ww\ yy$. Here the F_1 plants, $Ww\ Yy$, are white-fruited. They should produce four types of gametes, WY , Wy , wY , and wy , and the F_2 expected from a cross between two such F_1 plants is indicated in Fig. 40.

Three-fourths of the plants in this generation will evidently carry W and will thus appear white-fruited no matter what the rest of the genotype may be. Of the one-fourth which have no factor for white, however, three-fourths, or three-sixteenths of the whole, will carry yellow and will thus appear yellow; and one-fourth, or one-sixteenth of the whole, will not and will thus appear green. The occurrence of white, yellow, and green in the F_2 in approximately the ratio expected on the assumption (12:3:1) is actually realized in breeding experiments. The essential fact here is that W masks everything which is hypostatic to it, so that Y , which segregates quite independently of white, produces a visible effect only in that fraction of the F_2 which lacks W .

In Poultry (the 13:3 Ratio). It has already been noted that the white plumage of White Leghorn fowls is almost completely dominant over the colored plumage of black, barred, or other colored varieties. The white

plumage of some other white varieties, however, such as White Wyandottes or White Plymouth Rocks, has been found to be *recessive* to colored plumage and to be due to a gene distinct from that which produces the white of Leghorns. Experiment shows that White Leghorns contain a color gene and with it a gene which inhibits its expression. They are genetically colored birds which are unable to develop their true color. Denoting such an inhibitor by *I* and the color gene by *C*, the White Leghorn

P₁	White WW YY	Green ww yy		
F₁	White Ww Yy			
	WY	Wy	wY	wy
WY	WW YY White	WW Yy White	Ww YY White	Ww Yy White
Wy	WW Yy White	WW yy White	Ww Yy White	Ww yy White
wY	Ww YY White	Ww Yy White	ww YY Yellow	ww Yy Yellow
wy	Ww Yy White	Ww yy White	ww Yy Yellow	ww yy Green

FIG. 40. The 12:3:1 ratio. Checkboard showing the expected composition of the F₂ from a cross of a white-fruited squash plant which carries yellow, with a green-fruited one.

is *II CC* and the White Wyandotte is *ii cc*. A test of this hypothesis by crossing White Leghorns with White Wyandottes produces a curious result. The F₁ chickens from such a cross are white with small, dark flecks and resemble the F₁ birds produced by crossing White Leghorns with colored fowls. When these F₁ whites are bred together, however, white and colored chicks appear in F₂ in the proportion of about $13\frac{1}{16}$ white (or white with small, dark flecks) to $\frac{3}{16}$ colored. Although this ratio is not like that of any of the other dihybrid ratios which have been discussed, it may be explained in the same way as these with the additional assumption that *I* is epistatic to or hides the segregation of *C* (see Fig. 41).

The complications introduced by epistasis are comparable with those

produced by dominance, that is, two or more genotypes are indistinguishable in appearance. In cases of epistasis, however, there are always two or more genes involved, each of which affects the same part of the organism. This same condition occurred in cases of interaction such as that observed in comb shape in fowls, but in these latter *both* genes are expressed, producing a new or different condition of the part. In epistasis, on the contrary, the competition of two genes for expression in one part results in the

P_1		White (Leghorn) II CC		White (Wyandotte) ii cc	
F_1		White Ii Cc			
		IC	Ic	iC	ic
F_2	IC	II CC White	II Cc White	Ii CC White	Ii Cc White
	Ic	II Cc White	II cc White	Ii Cc White	Ii cc White
	iC	Ii CC White	Ii Cc White	ii CC Colored	ii Cc Colored
	ic	Ii Cc White	Ii cc White	ii Cc Colored	ii cc White

FIG. 41. The 13:3 ratio. Checkerboard showing the expected composition of the F_2 from a cross between two varieties of fowls, one with dominant white plumage and one with recessive white plumage.

apparent triumph of one and the suppression of the other, so that the original traits are recovered but in modified ratios.

Duplicate Genes. An important modification of the dihybrid ratio occurs when either one or the other of two independent genes produces the same or a closely similar effect. Genes with the same expression are known as duplicate genes. Their discovery pointed the way to an explanation of some of the complex cases in which many genes with similar or identical effects each contributes to a variable character such as size.

In Bursa (the 15:1 Ratio). One of the simplest instances of duplicate

factors is involved in the inheritance of capsule or pod form in the shepherd's-purse Bursa, as reported by G. H. Shull. One race of this species has characteristically triangular capsules, whereas in another they are ovoid or top-shaped. When these two types are crossed, the F_1 plants all have triangular capsules, the dominance of this shape being complete. In the F_2 , however, there are ordinarily found to be about 15 plants with typical triangular capsules to every 1 with ovoid capsules. This F_2 ovoid plant breeds true in subsequent generations, whereas of the F_2 triangular plants some breed true, others produce triangular and ovoid plants in the ratio of 3:1, and others produce these types in the ratio of 15:1. Remembering that, where parents differing in two genes are crossed, the double recessive type appears in only one-sixteenth of the F_2 progeny (as opposed to one-fourth in a monohybrid cross), the hypothesis at once suggests itself that the triangular capsule of Bursa differs from the ovoid by two dominant duplicate genes or by either one of them alone and that the ovoid type is due to the recessive alleles of both of these. The 15:1 ratio is thus still another modification of the 9:3:3:1 ratio, the first three terms here being indistinguishable from each other. Representing the two genes by T_1 and T_2 , the genotypes and phenotypes of the F_2 are shown in the checkerboard in Fig. 42.

The hypothesis of duplicate factors not only explains this peculiar ratio but makes it easier to understand the differences in breeding behavior which have been found to exist between the various triangular types occurring in F_2 . Seven plants out of sixteen (those which are homozygous for either T_1 or T_2 or both) should breed true to triangular capsules in later generations. Four plants have only one of the genes represented and that in a heterozygous condition ($T_1t_1 t_2t_2$ or $t_1t_1 T_2t_2$), so that these plants may be expected to produce offspring about three-fourths of which have triangular capsules and one-fourth ovoid ones. The remaining four are heterozygous for both genes ($T_1t_2 T_2t_1$) and should thus produce offspring, when inbred, in about the ratio of 15:1, just as does the F_1 hybrid. These expectations have been borne out by actual breeding tests.

In this case T_1 and T_2 each is dominant to its allele and shows no cumulative effect. The analysis of other cases in which dominance is lacking and the different duplicate genes reinforce each other to produce a cumulative effect laid the foundation for the Multiple-factor theory and will be discussed in Chapter VI.

Analysis of Coat Color in Mice. A thorough study of the variations in a group of related characters in any organism will usually reveal an intricate series of interactions between the component genes. As an example we shall choose the house mouse, for in this animal a large number of spontaneous variations have provided the opportunity for a genetic analysis

of the genes affecting coat color. Many such genes have been studied and their interrelationships made out. *C* is the fundamental color gene, necessary for the production of any pigment in the coat. Another gene, *A*, or gray, determines the development of the agouti pattern. Its recessive allele, *a*, is present in the nonagouti mice, such as blacks or browns. Still

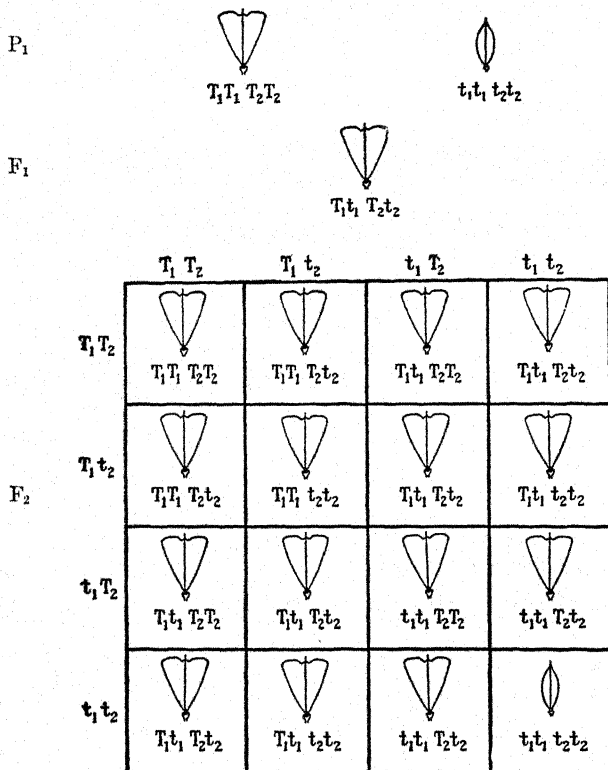


FIG. 42. The 15:1 ratio. Checkerboard showing the expected composition of the F_2 from a cross between a type of shepherd's-purse (*Bursa*) with triangular capsules (homozygous for two duplicate factors) and a type with top-shaped capsules. (After G. H. Shull.)

another, *B*, governs the development of black pigment and is dominant over its allelic condition of brown or chocolate, *b*. Many varieties are spotted with white in a blotched, or piebald, pattern, and such mice contain a gene, *s*, which is recessive to self-, or solid, color, *S*. Another gene, *d*, brings about a clumping of the black and brown pigment granules in the hairs and makes these colors appear faded, or *dilute*, as opposed to the normal fully pigmented form, *D*. Another gene reduces the amount of

black and brown pigment in the fur, giving it a pale and washed-out appearance, and also reduces the pigment in the iris, making the eyes appear reddish or pink like the eyes of albinos. This gene, which is called pink eye (*p*) from its most noticeable effect, is recessive to the normal dark-eyed, intense-colored condition, *P*. These genes all segregate sharply and may occur in any combination. There are also several other genes affecting coat color which will be omitted for the sake of simplicity. Some of these combinations result in characters which are distinctive and have been given names of their own. Thus the nonblack agoutis are called "cinnamon," or brown agouti; the dilute blacks, "blue"; the dilute browns, "silver fawn"; and so on. Table XIV lists these various gene combinations, together with the type of coat color produced by each.

All of these types are recessive to the wild coat and appear to have arisen from it by mutation of one or more genes. Thus at any time the wild type may be reconstituted by bringing into combination all of the alleles of the genes which are responsible for these new types. *In fact, the wild coat color itself is found to depend on the presence and interaction of all of the genes named.* Thus, in order to produce the agouti pattern there must be present the genes for color (*C*), agouti (*A*), black (*B*), dark eye (*P*), dense color (*D*), and solid color (*S*). With regard only to these genes the genotype of the wild mouse may be written *AA BB CC DD PP SS*. These genes all show essentially complete dominance, so that their heterozygous condition will give the same result as is produced by the homozygous form here given. Thus an animal with the genotype *AaBbCcDdPpSs* would also be agouti in appearance. The genes named do not include all that are known, nor is it believable that more than a small sample of the genes affecting coat color in mice has been studied. Were knowledge complete, it is probable that the list of genes necessary for the production of the agouti pattern would be much longer and that the letters of the alphabet would be exhausted in attempting to write the genotype of the wild mouse. Here, then, is a clear and convincing example of gene interaction. In order that the apparently simple pattern characteristic of wild house mice may be developed, there must be present at least six genes (probably many more) each of which has a definite effect on coat color. If any single gene is missing or changed, a coat pattern differing more or less widely from the wild type results.

This type of gene interaction is not exceptional but is found whenever numerous variations in a single aspect of the organism are carefully analyzed. Such analyses have been made for several groups of characters in maize. More than a dozen series of genes affecting chlorophyll development in this plant are known. The normal color is green, which results from the combined action of all of the normal genes. If one of these genes

TABLE XIV. INTERACTION OF GENES FOR COAT COLOR IN MICE

Genes					Gametic formula	Phenotype		
C	A	B	D	P	S	CABDPS	Wild-type agouti	
				s	CABDPs	Spotted agouti		
			p	S	S	CABDpS	Pink-eyed agouti	
				s	CABDps	Pink-eyed, spotted agouti		
			d	P	S	S	CABdPS	Dilute agouti
					s	CABdPs	Spotted, dilute agouti	
				p	S	S	CABdpS	Pink-eyed, dilute agouti
					s	CABdps	Pink-eyed, spotted, dilute agouti	
		b	D	P	S	CAbDPS	Cinnamon	
				s	CAbDPs	Spotted cinnamon		
				p	S	S	CAbDpS	Pink-eyed cinnamon
					s	CAbDps	Pink-eyed, spotted cinnamon	
			d	P	S	S	CAbdPS	Dilute cinnamon
					s	CAbdPs	Spotted, dilute cinnamon	
				p	S	S	CAbdpS	Pink-eyed, dilute cinnamon
					s	CAbdps	Pink-eyed, spotted, dilute cinnamon	
	a	B	D	P	S	CaBDPS	Black	
				s	CaBDPs	Spotted black		
			p	S	S	CaBDpS	Pink-eyed black	
				s	CaBDps	Pink-eyed, spotted black		
			d	P	S	S	CaBdPS	Dilute black
					s	CaBdPs	Spotted, dilute black	
				p	S	S	CaBdpS	Pink-eyed, dilute black
					s	CaBdps	Pink-eyed, spotted, dilute black	
		b	D	P	S	CabDPS	Brown	
				s	CabDPs	Spotted brown		
				p	S	S	CabDpS	Pink-eyed brown
					s	CabDps	Pink-eyed, spotted brown	
			d	P	S	S	CabdPS	Dilute brown
					s	CabdPs	Spotted, dilute brown	
				p	S	S	CabdpS	Pink-eyed, dilute brown
					s	Cabdps	Pink-eyed, spotted, dilute brown	
c with any other genes					c	Albino, may be of any of 32 genotypes above		

such as *W* mutates to a recessive allele *w*, the seedling is albinotic, virtually without any chlorophyll, and being unable to carry on photosynthesis it soon dies. There are at least 15 different genes susceptible to this type of change, which means that there are at least 15 genetically different types of albinos. In another group of recessive mutants known as lutescents only yellow pigment develops; in another group are more than 20 recessive genes each of which when homozygous produces virescent seedlings which are albinotic but eventually develop enough chlorophyll to keep them alive. Other recessive genes are responsible for pale green color (10 known), zebra-stripping (4 known), piebald spotting (4 known), golden color (4 known), yellow-green color (3 known), yellow striping (2 known), fine white striping (3 known), and many other modifications of chlorophyll composition and arrangement. It must be true therefore that the normal development of chlorophyll in maize depends on interaction among at least 75 different genes. If any one of these changes by mutation from the normal to the recessive allele, some essential step in the interaction fails and the chlorophyll is absent or deficient in some way. Similarly each of another large group of genes conditions some step in the development of anthocyanin pigments, while the normal starchy endosperm of the kernel of field corn (dent or flint) depends on the interaction of more than 30 different genes. The results of the analysis of such cases as those described above suggest that many of the characters of organisms are the end products of long chainlike series of related steps, $a \rightarrow b \rightarrow c \rightarrow d \rightarrow n$. Separate genes seem to affect separate steps so that if the gene affecting step *b* does not perform its task, then *c* and all later steps which depend upon it cannot take place and the character, such as normal chlorophyll, cannot appear. Other evidence for this view of the mechanism of gene interaction will be presented in a later chapter. Whatever the means by which the genes interact, it may be accepted as a general rule that the hereditary characters of a plant or animal depend upon the balanced cooperation of a large number of genes.

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PROBLEMS

125. Calculate the goodness of fit of the actual ratios in Table XIII to expected ratios of 1:1 (first line), 3:1 and 2:1 (second line). What conclusions can be drawn from the P values?

126. A cross of two yellow mice gave the following result: 24 yellow, 12 black, 12 albino. What were the genotypes of the parents?

127. In mice what will be the appearance of the offspring from the following crosses (A^Y = yellow):

$$\begin{aligned} CC A^Y A BB &\times CC A^Y A bb \\ Cc A^Y A BB &\times Cc A^Y A Bb \\ cc A^Y A BB &\times Cc A^Y A BB \end{aligned}$$

128. If a large population of yellow mice is allowed to mate at random, permitting matings only among members of the same generation, what would be the expected frequency of yellows and nonyellows in the F_1 - F_3 generation? Plot graphically the changes in the frequency of the gene A^Y during this period.

129. In *Drosophila* crosses of Dichaete winged flies \times Dichaete always give two-thirds Dichaete to one-third normal winged offspring. Dichaete \times normal gives one-half Dichaete and one-half normal. How would you explain these results?

130. In poultry the following results were obtained:

	Progeny	
	Short-legged	Normal
(a) Short-legged \times short-legged.....	1,972	955
(b) Short-legged \times normal.....	1,676	1,661

Explain these results, giving genotypes of animals involved. Explain how you would test your hypothesis (a) by statistical methods; (b) by obtaining what additional facts?

131. From present knowledge of the inheritance of thalassemia what practical steps could be taken to reduce the frequency of this gene?

132. The frequency of children homozygous for a lethal gene (juvenile amaurotic idiocy) is about 1 per 25,000 (.00004) in Sweden. What should be the proportion of heterozygotes in this population? How could heterozygotes be detected?

133. Can pure stocks of the following be produced: yellow mice; walnut-combed fowls; blue Andalusian fowls? Explain.

134. How could you most easily distinguish the various genotypes among the walnut-combed F_2 birds in the checkerboard in Fig. 37?

135. Can you suggest a chemical or physical explanation for the multiple effects of a single factor such as Mendel found in purple-flowered peas?

136. In mice how would you recognize a *recessive* trait which has also a lethal effect before birth?

137. Describe one or more instances in man where the expression of an inherited defect has been modified by training or environment.

Note. In poultry the genes for rose comb, R , and pea comb, P , if present together, produce walnut comb. The recessive alleles of both, when present together in a homozygous condition, produce single comb.

138. What will be the comb character of the offspring of the following crosses, in which the genotypes of the parents are given:

$$\begin{array}{l} Rr Pp \times Rr Pp \\ RR Pp \times rr Pp \\ rr PP \times Rr Pp \end{array}$$

$$\begin{array}{l} Rr Pp \times Rr pp \\ Rr pp \times rr Pp \\ Rr pp \times Rr pp \end{array}$$

Note. In the following five questions, all of which concern comb form in poultry, determine the genotypes of the parents:

139. A rose crossed with a walnut produces offspring three-eighths of which are walnut, three-eighths rose, one-eighth pea, and one-eighth single.

140. A walnut crossed with a single produces offspring one-fourth of which are walnut, one-fourth rose, one-fourth pea, and one-fourth single.

141. A rose crossed with a pea produces six walnut and five rose offspring.

142. A walnut crossed with a single produces one single-comb offspring.

Note. In poultry, feathered shanks, F , are dominant over clean, f ; and the white plumage of Leghorns, I , is dominant over black, i .

143. What will be the appearance of the offspring of the following crosses, in which the genotypes of the parents are given:

$$\begin{array}{l} ff Rr Pp \times Ff Rr pp \\ Ff ii Rr pp \times ff II Rr Pp \end{array}$$

144. A feather-shanked, rose-comb bird crossed with a clean-shanked, pea-comb one produces 25 feathered, pea offspring; 24 feathered, walnut; 26 feathered, rose; and 22 feathered, single. What are the genotypes of the parents?

145. A breeder has a homozygous race of feather-legged, black, rose-comb birds and another of clean-legged, white, pea-comb ones. He wants a race of black birds which have clean legs and walnut combs. What proportion of the F_2 raised from a cross between these two races will be what he desires in *appearance*? What proportion of these birds will be homozygous for the desired characters?

146. If the disk-fruited F_1 squash plants resulting from a cross of sphere \times sphere (p. 102) are crossed with elongate-fruited plants, what will be the fruit shape of their offspring?

147. A certain disk-fruited squash plant crossed with a spherical-fruited one produces offspring of which three-eighths are disks, one-half spheres, and one-eighth elongates. What are the genotypes of the parents?

Note. In sweet peas, genes C or P alone produce white flowers, the purple color being due to the presence of both these factors.

148. What will be the flower color of the offspring of the following crosses, in which the genotypes of the parents are given:

$$\begin{array}{l} Cc Pp \times cc Pp \\ Cc Pp \times Cc PP \end{array}$$

$$\begin{array}{l} cc Pp \times CC pp \\ Cc pp \times cc Pp \end{array}$$

149. In the checkerboard (Fig. 38) what will be the flower color of the offspring of each of the nine purple-flowered plants if selfed?

Note. In the following three crosses of sweet peas what are the genotypes of the parents?

150. A white-flowered plant crossed with a purple produces offspring of which three-eighths are purple and five-eighths white.

151. A purple-flowered plant crossed with a white one produces offspring of which one-half are purple and one-half white.

152. A white-flowered plant crossed with another white produces offspring of which three-fourths are white and one-fourth purple.

153. In a recent study of a Chinese population three classes of persons were found in respect to tongue movements as follows:

615 could roll the tongue, curling it from side to side, but could not fold it back on itself.

34 could both roll and fold the tongue.

394 could neither roll nor fold the tongue.

None were found who could fold but not roll the tongue.

Other evidence makes it probable that rolling ability (R) is dominant to non-rolling (rr), folding ability (ff) recessive to nonfolding. Explain the above facts, giving genotypes.

Note. In maize, C and R are both necessary for the production of red aleurone color, the absence of either resulting in white aleurone. If P is present in addition to C and R , the aleurone is purple, but P has no effect in the absence of either C or R or both.

154. In maize, what is the aleurone color of the offspring of the following crosses, the genotypes of the parents being given:

$$Cc Rr pp \times cc Rr Pp$$

$$CC rr Pp \times Cc Rr pp$$

$$cc RR Pp \times Cc Rr pp$$

$$Cc Rr Pp \times Cc Rr Pp$$

Note. In the following three questions, all of which refer to aleurone color in maize, find the genotypes of the parents.

155. A purple plant crossed with a white produces offspring of which one-eighth are purple, one-eighth red, and three-fourths white.

156. A purple plant crossed with a red produces offspring of which nine thirty-seconds are purple, nine thirty-seconds red, and seven-sixteenths white.

157. A purple plant crossed with a white produces offspring of which three-eighths are purple and five-eighths white.

Note. The effect of the *C*, *A*, *B*, *D*, *P*, and *S* and their recessive alleles on coat color in mice is as follows (see Table XIV): *C*, colored; *c*, albino; *AB*, black agouti (wild type); *Ab*, cinnamon (brown agouti); *P*, normal dark eyes; *p*, pink eyes; *aB*, black; *ab*, brown; *D*, normal dark color; *d*, dilute color; *S*, solid color, or self color; *s*, spotted with white.

158. In mice what will be the coat color of the offspring of the following crosses, in which the genotypes of the parents are given:

$$Cc Aa Bb \times CC aa Bb$$

$$Cc Aa BB Dd \times cc Aa Bb Dd$$

$$CC aa Bb dd Pp \times Cc aa Bb Dd pp$$

$$CC AA BB Dd Pp SS \times Cc aa Bb DD Pp ss$$

Note. In the following five crosses, which deal with coat color in mice, find the genotypes of the parents.

159. An agouti animal crossed with another agouti produces offspring of which nine-sixteenths are agouti, three-sixteenths black, three-sixteenths cinnamon, and one-sixteenth brown.

160. A cinnamon animal crossed with an albino produces offspring of which three-eighths are agouti, one-eighth black, and one-half albino.

161. A black animal crossed with an agouti produces offspring of which nine thirty-seconds are agouti, nine thirty-seconds black, three thirty-seconds cinnamon, three thirty-seconds brown, and one-fourth albino.

162. A dilute agouti animal crossed with a pink-eyed, spotted black produces three agouti offspring, one spotted agouti, two dilute agouti, two dilute, spotted agouti, four cinnamon, one spotted cinnamon, two dilute cinnamon, and four albinos.

Note. In summer squashes the gene for white fruit color, *W*, is epistatic to that for yellow, *Y*; *WY* and *Wy* plants are white, *wY* plants yellow, and *wy* plants green.

163. What is the color of the fruit in the offspring of the following crosses, the genotypes of the parents being given:

$$Ww Yy \times Ww yy$$

$$ww YY \times Ww yy$$

$$Ww yy \times ww Yy$$

Note. In the following three questions, which deal with fruit color in squashes, find the genotypes of the parents:

164. A white plant crossed with a yellow one produces offspring of which one-half are white, three-eighths yellow, and one-eighth green.

165. A white plant crossed with a green one produces offspring of which one-half are white and one-half yellow.

166. A white plant crossed with another white one produces offspring of which three-fourths are white, three-sixteenths yellow, and one-sixteenth green.

167. What will be the plumage color of the offspring of the following crosses in poultry, the genotypes of the parents being given (p. 110):

$$\begin{array}{l} Ii Cc \times ii Cc \\ II cc \times ii cc \end{array}$$

$$\begin{array}{l} ii Cc \times Ii CC \\ Ii cc \times ii Cc \end{array}$$

168. From the data on inheritance of capsule shape in the shepherd's-purse, calculate the expected results in F_3 from self-fertilizing each of the 15 F_2 types with triangular capsules in Fig. 42.

169. In each of two different strains of maize, plants have been found which, when selfed, produce about three-fourths normal green and one-fourth lethal white ("albino") seedlings. If two such albino-producing plants, one from each strain, are crossed, the F_1 is found to be all green, but certain of the F_2 populations are approximately nine-sixteenths green and seven-sixteenths white. Explain, giving genotypes.

170. In maize, plant A when crossed with plant B produced 255 green and 89 white offspring but when selfed produced 153 green and 118 white offspring. What are the genotypes of these two plants? What should plant B produce when selfed?

171. A green maize plant when selfed produces about fifteen-sixteenths green and one-sixteenth white (lethal) seedlings. Explain.

172. In maize, the scutellum develops color only when certain aleurone genes and any two of the three genes S_2 , S_3 , and S_4 are present. Thus, $S_2S_2S_3S_3S_4S_4$ and $s_2s_2S_3S_3S_4S_4$ have colorless scutellum. What ratios in respect to scutellum color are to be expected in F_2 from

(a) colorless \times colorless ($S_2S_2S_3S_3S_4S_4 \times s_2s_2S_3S_3S_4S_4$)

(b) colored \times colored ($S_2S_2S_3S_3S_4S_4 \times s_2s_2S_3S_3S_4S_4$)

Would you classify the interaction of these genes as duplicate or complementary?

173. A given F_2 population consists of 1,250 B and 350 b individuals. Does this fit better a 3:1 or a 13:3 segregation? Decide this by calculating S.E._d for each ratio.

174. By calculating χ^2 and P determine whether the following F_2 population fits better a 3:3:1:1 ratio or a 27:21:9:7 ratio:

AB	Ab	aB	ab
285	245	90	84

On the basis of this test, what hypothesis would you adopt concerning the genotypes of the parents and the action of the genes involved, assuming that they affect aleurone color in maize?

CHAPTER VI

THE MULTIPLE-FACTOR HYPOTHESIS

In the period immediately following 1900, the use of Mendel's methods and principles led to a satisfactory interpretation of the inheritance of many of the differentiating characters of animals and plants which were relatively little affected by the environment and could be most accurately described and classified. Even complex cases of gene interaction, such as some of those described in Chapter V, could be easily analyzed when the phenotypes concerned could be separated into sharp alternative categories.

However, not all individual differences can be readily classified in this way. Variations such as those in size, complexion, and mental ability in men, the traits which are most important economically in domestic animals and plants, such as yield of fruits or seeds or eggs or amount of meat or milk produced, and many of the differences among related species in nature usually do not fall into a few alternative classes but into many small, continuously intergrading ones. It is a matter of common observation that crosses between large and small types or as in man between black- and white-skinned people usually result in F_1 individuals which are intermediate between the parents, followed in F_2 and later generations by a wide array of variant types, among which the parental type may be rare or not represented at all. The question which began to be asked as soon as the significance of Mendel's discovery was recognized was whether the inheritance of such quantitative variations can be explained by Mendel's principles.

One answer to this question had, of course, been given by Mendel himself, for one of the traits on the analysis of which he founded his theory was, in fact, a "quantitative" one, the difference between tallness and dwarfness in peas. In this case the contrasted classes were so distinct that no overlapping occurred between tall and dwarf classes so that no measurements were needed. It is thus not the "quantitative" nature of such variations which set them apart from those described in "qualitative" terms, but the transgressive or apparently continuous way in which the variations must be described. As a matter of fact, the same character, such as size, which in a population of microorganisms may be treated as quantitative at one magnification, may, at another, be classified into discontinuous categories and treated qualitatively.

Moreover, dimensional traits are often more sensitive to environmental

modification, since the amount of growth depends on temperature, light, supply of nutrients, and similar fluctuating factors which cause continuous variations. As Johanssen had shown, variability from these causes is superimposed upon genotypic variability in organisms other than those belonging to the same pure line.

It is not surprising, then, that for technical reasons the genetic analysis of transgressively varying characters has not kept pace with other applications of Mendelian analysis. Nevertheless, by 1910 the essential observations had been made which showed that Mendel's principles provide the basic genetic mechanism for understanding the inheritance of transgressive variations.

The Multiple-factor Hypothesis. The absence of clear-cut segregation into definite and readily recognizable classes, which is the main difference between the inheritance of typical quantitative characters and that of the ones which we have chiefly considered thus far, may perhaps best be explained by a study of the manner of inheritance of one of those traits which is sometimes a simple qualitative one but at other times behaves in a more complex manner. In a previous chapter (p. 112) the operation of *duplicate* genes has been described. The case of kernel color in wheat, as described by Nilsson-Ehle, is particularly illuminating. Here red is not completely dominant over white, but the hybrids may show intermediate intensities of color. In some crosses of red with white a simple ratio of $\frac{3}{4}$ red: $\frac{1}{4}$ white is found, indicating a single gene difference, but some of the reds are as dark as the red grandparent and others resemble the F_1 in being less intense. In other crosses a ratio of $\frac{15}{16}$ red: $\frac{1}{16}$ white is found in the F_2 . In this case there are a few which are as dark red as the red grandparent, a considerable number which are of about the same shade as the F_1 , and some which are even paler than the F_1 . The conclusion seems obvious (and is supported by a study of F_3 generations raised from these various types) that there are here two pairs of genes for red, neither completely dominant over white and either capable of producing red kernels, but that the four members of these pairs are cumulative in their effect, the dark reds being due to the presence of four genes, the next in intensity to three, those like the F_1 to two, and the pale reds to but one. The checker-board in Fig. 43 shows the genotypes of the F_2 plants, with the number of red genes possessed by each class. Only 1 of the 15 reds has all four genes and is thus dark red; 4 have three genes; 6 have two; 4 have one; and one-sixteenth of the whole population has none.

In still other cases Nilsson-Ehle found that $\frac{63}{64}$ of the F_2 plants were redkerneled and only $\frac{1}{64}$ white, a condition which suggested that *three* independent genes were involved. Here the range in intensity of the red was even more marked, as was to be expected. If the red parent is repre-

sented by $R_1R_1R_2R_2R_3R_3$ and the white by $r_1r_1r_2r_2r_3r_3$, the F_1 ($R_1r_1 R_2r_2 R_3r_3$) should evidently be essentially uniform and intermediate between the parents in color, both of which expectations are realized. In the F_2 there should also be a marked increase in the range of color types. The relative proportions of the various groups may readily be calculated. A few individuals ($1/64$) may be expected to have six genes for red, others ($6/64$) five,

P_1	$R_1R_1 R_2R_2$ Red	$r_1r_1 r_2r_2$ White				
F_1	$R_1r_1 R_2r_2$ Red					
	R_1R_2	R_1r_2	r_1R_2	r_1r_2		
F_2	R_1R_2	$R_1R_1 R_2R_2$ Red	$R_1R_1 R_2r_2$ Red	$R_1r_1 R_2R_2$ Red	$R_1r_1 R_2r_2$ Red	
	R_1r_2	$R_1R_1 R_2r_2$ Red	$R_1r_1 r_2r_2$ Red	$R_1r_1 R_2r_2$ Red	$R_1r_1 r_2R_2$ Red	
	r_1R_2	$R_1r_1 R_2R_2$ Red	$R_1r_1 R_2r_2$ Red	$r_1r_1 R_2R_2$ Red	$r_1r_1 R_2r_2$ Red	
	r_1r_2	$R_1r_1 R_2r_2$ Red	$R_1r_1 r_2r_2$ Red	$r_1r_1 R_2r_2$ Red	$r_1r_1 r_2r_2$ White	

FIG. 43. Diagram showing the result of a cross between a red-kerneled and a white-kerneled wheat, where the red color is due to the operation of either or both of two genes, R_1 and R_2 . The intensity of the red color is indicated by the density of the crosshatching.

others ($15/64$) four, others ($20/64$) three, others ($15/64$) two, others ($6/64$) one, and a few ($1/64$) none at all (resulting in white kernels). Although it is not always possible to distinguish these six classes of reds by their appearance, it is evident that there are *few* of either extreme type and *many more* of the intermediate grades, and segregation in later generations indicates the essential correctness of this three-gene hypothesis.

In the uniform but intermediate character of the F_1 and the marked increase in variability of the F_2 , red kernel color of wheat in this case be-

haves essentially like an ordinary quantitative character. That the nature of its inheritance is truly Mendelian is proved not only by an analysis of the results of this cross but by a comparison between it and the obviously simpler results (3:1 and 15:1 segregations) for the same character in related races. From a study of such cases as this Nilsson-Ehle in 1908 proposed the multiple-factor hypothesis for the inheritance of quantitative characters, and this was quickly extended by East (1910). This assumes that there is a *series of independent genes* for a given quantitative trait and that these genes are cumulative in their effect. Dominance is ordinarily absent, the F_1 appearing as a blend of the characters of the two parents. If instead of a unit of color (as in wheat kernels) each gene should contribute a unit of height, weight, or other typically quantitative trait, the relations be-

TABLE XV. FREQUENCY DISTRIBUTIONS EXPECTED IN F_2 POPULATIONS FROM CROSSES OF PARENTS DIFFERING IN A DIMENSIONAL TRAIT BY 12 UNITS WHEN THE DIFFERENCE IN THE PARENTS IS DETERMINED BY ONE, TWO, THREE, AND SIX PAIRS OF INDEPENDENT GENES, WITH EQUAL ADDITIVE EFFECTS

Gene pairs	Class Centers													<i>n</i>	
	P ₁			F ₁								P ₂			
	101	102	103	104	105	106	107	108	109	110	111	112	113		
1	1,024						2,048							1,024	4,096
2	256			1,024			1,536			1,024				256	4,096
3	64		384			960	1,280		960		384			64	4,096
6	1	12	66	220	495	792	924	792	495	220	66	12		1	4,096

tween parents, F_1 , and F_2 , would be essentially like those just described for kernel color.

A given size difference between two races of plants or animals may be due to one, two, or several such gene differences. A comparison of the kinds of populations to be expected in F_2 from parents whose differences in a quantitative trait such as size are due to 1, 2, 3, . . . , 6 pairs of independent genes with equal and cumulative effects and without dominance are shown in Table XV. The frequencies will be recognized as proportional to the coefficients in the expansion of $(a + b)^n$ for the even powers from 2 to 12 corresponding to the number of alleles contributing to the total result. As the number of alleles increases, the relative frequency of the parental types recovered in F_2 declines sharply and the different F_2 phenotypes are spread much more evenly over the range, approaching the type of continuous distribution which is obtained from the expansion of the binomial and from the measurements of a continuously variable trait such as size in actual F_2 populations. It was, in fact, upon this very resemblance that the multiple-

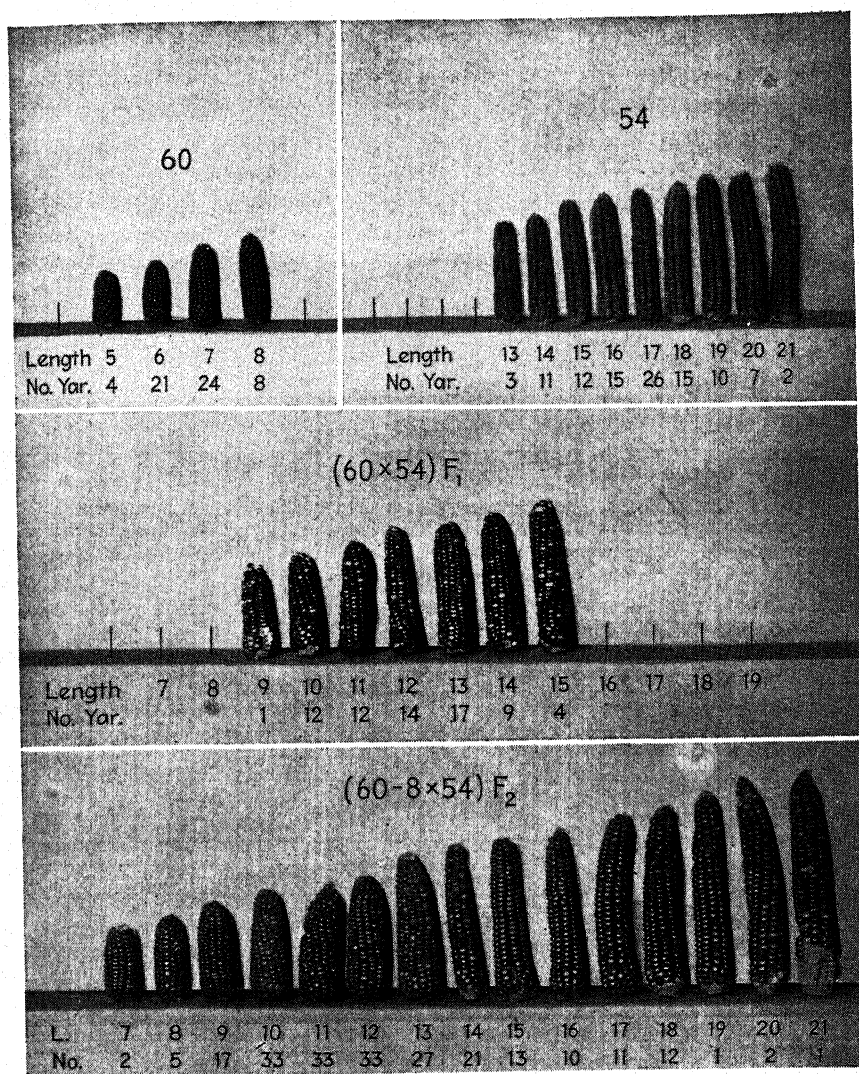


FIG. 44. The inheritance of ear length in maize, as shown by the results of crossing a short-eared variety of popcorn with a long-eared variety of sweet corn. Ears showing the range in length of the parent types are pictured above, with the F_1 and F_2 generations below. (From East.)

factor theory was first applied to such characters. Two examples will illustrate the application of the theory.

In his study of the inheritance of ear length in maize, East worked with two races or varieties: one, long-eared Black Mexican sweet corn, and the other, short-eared Tom Thumb popcorn. In Fig. 44 are shown the parental types and the F_1 and F_2 generations following a cross between them. The

ears have been arranged in classes differing by 1 cm. in length, and under each class is given the number of individuals in that class. Thus in the F_1 there was 1 plant in which the ear was 9 cm. long, there were 12 in which it was 10 cm., and so on. Each of the pure types evidently varied somewhat in ear length, presumably because of environmental differences, and the F_1 shows about the same degree of variability, although it is essentially intermediate between the two parental types. In the F_2 , however, there is a much greater range, from plants with ears as short as the shorter eared grandparent to those with ears as long as the longer eared grandparent. There are relatively few of these extreme types and a relatively large number of those which have intermediate ear length essentially like that of the

TABLE XVI. INHERITANCE OF QUANTITATIVE DIFFERENCES IN PIED SPOTTING
(From Dunn and Charles)

	Per cent of Dorsal White																					
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
P ₁ Line 118 (F ₇₋₁₄).....		7	8	9	35	2	1															
P ₁ Line 19 (F ₇₋₈).....																		1	9	29	134	
F ₁ Line 19 × Line 118.....					1	1	4	3	3	10	7	9	9	7	1	1						
F ₂ Line 19 × Line 118.....			1	2	6	15	10	12	14	19	27	29	28	19	34	21	14	2	3	2		
BC F ₁ × Line 19.....														5	16	33	24	15	10	9	12	
P ₁ Line 190a (F ₁₀₋₁₇).....			3	10	22	23	31	27	28	15	5											
P ₁ Line 19 (F ₇₋₈).....																		1	9	29	134	
F ₁ Line 190a × Line 19.....													1	5	4	1						
F ₂ Line 190a × Line 19.....									2	5	1	7	16	29	45	10	3	2	3	2		

F_1 . This situation is similar in its essentials to that of kernel color in wheat, and although segregation into a series of distinct classes is impossible to demonstrate, the marked increase in variability of the F_2 as compared with the F_1 finds its simplest explanation in the independent segregation of a series of "multiple factors" each affecting ear length and all of them cumulative in their effect.

In several other analyzed cases, inbreeding different F_2 types results, in F_3 , in populations differing in the average size, some like one parent, some like the other parent, while others are intermediate, showing that the size differences are due to different gene combinations.

In a population of mice homozygous for the gene for pied spotting, *ss*, there may be continuous variation from animals with only a small white spot on the belly to those with an entirely white coat, having pigment only in the eyes (Fig. 45). By inbreeding and selection it is possible to produce strains which regularly differ in the range of spotted types which they produce. The results of crossing some of these types are shown in Table

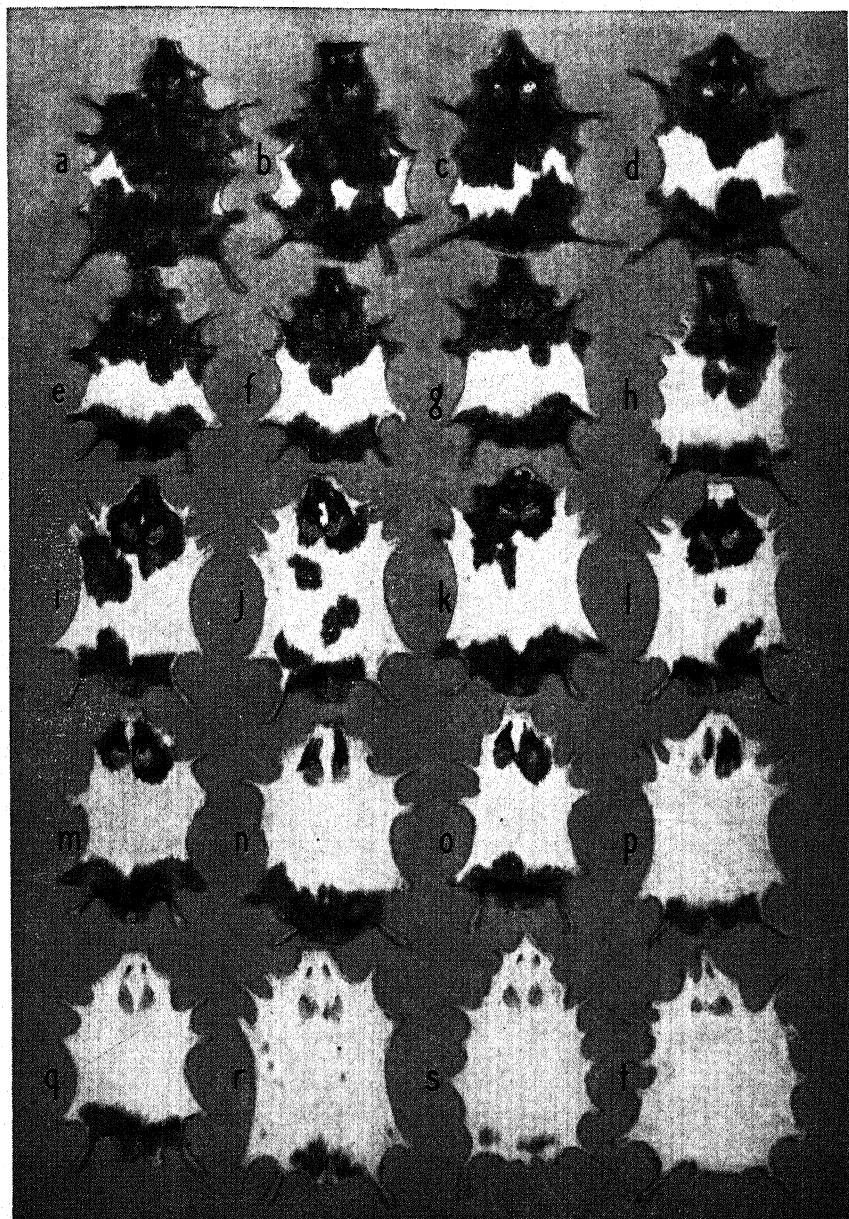


FIG. 45. Variation in the pied (piebald) spotting in laboratory strains of mice, due to multiple factors. (From Dunn & Charles.)

XVI. As in the cross of corn varieties differing in ear length, the F_1 is intermediate, while F_2 is more variable, and a few individuals within the range of the parent types are recovered. In a backcross of F_1 with animals of the nearly all white stock, the parental type was recovered with about the frequency that would be expected if three or four genes with quantitative effects were segregating in F_1 . It is evident from the results that the lighter and darker spotted strains differ by several nondominant genes $s_1 \dots s_n$, whose effects cumulate with each other and with those of ss , the pied gene common to all of these strains. This conclusion was later confirmed by separating the $s_1 \dots s_n$ genes from s and analyzing them in outcrosses. Similar evidence has been obtained for the multiplicity of white-spotting factors in guinea pigs, in rats, and in rabbits.

The applicability of the multiple-factor theory to the two cases of transgressive variation described above has been attested by the similarity between the results obtained from crosses between phenotypes differing in a quantitative character and those expected from the segregation and recombination of genes with quantitative effects. It has been similarly applied to a large number of quantitative differences in general size, yield, flowering time, and similar variations, and the evidence is good that in many cases these differences are due to several, even to many, genes with small effects.

Race Differences. The results of crossing species and varieties of plants and animals and interracial hybridization in man likewise indicate that many gene differences, some with large and some with small effects, are concerned in causing the differences between the parental types. It is common knowledge that hybrids between dark and light races, as between Europeans and Negroes, are intermediate in color. When such mulattoes intermarry, their children, corresponding to an F_2 generation, are much more variable than either parent race or the F_1 . In skin color they vary from one grandparental extreme to the other. Children as light as the white type and as dark as the Negro are rather infrequent. It has been estimated that whites and Negroes differ by at least two genes for color of skin. If this is so, we should expect not more than $1/16$ white and $1/16$ black children among the offspring of mulattoes. As a matter of fact, variation in skin color is so continuous that it would be very difficult to separate such children into only the five classes called for by a two-gene hypothesis, and there is no doubt that environmental variation, and the effects of minor modifying genes from particular white or Negro parents, would generally obliterate any sharp distinctions between the classes in large populations. It is very probable that considerably more than two genes govern the inheritance of skin color.

When other traits in addition to skin color are followed, such as hair form and length and shape of nose, lips, face, and skull, the variability and re-

combination observed in the descendants of interracial marriages may be taken as evidence that these characters also are influenced by multiple genes. One is led to suppose that Negroes and whites differ by quite a large number of genes, and considering that they were geographically separated for long ages and unable to exchange genes, this is not at all surprising.

Similar results are obtained after crossing of geographically separated races of plants and animals. Northern races or species often flower earlier than related varieties from farther south even when grown in the same environment. Mendel crossed an early- and a late-flowering variety of peas and found that F_1 flowered at an intermediate date, but he did not com-

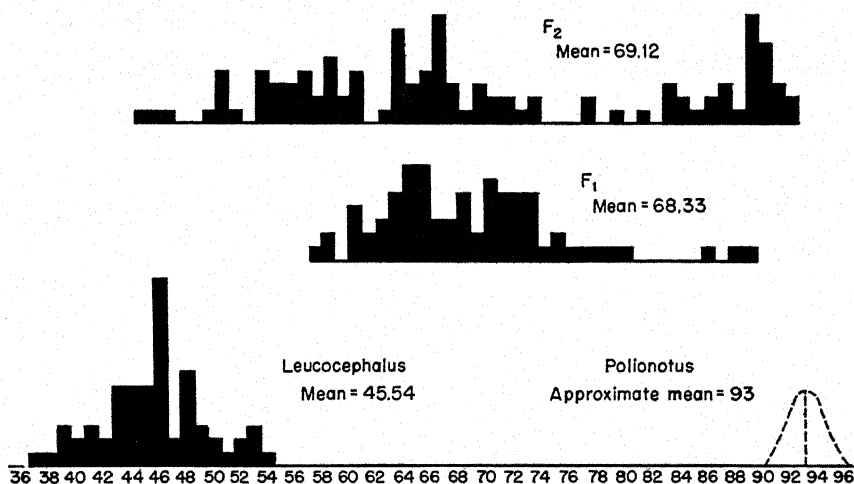


FIG. 46. Inheritance of extent of colored area in a cross between two geographic races of deer mice, a light race (*Peromyscus polionotus leucocephalus*) and a dark race (*P. polionotus polionotus*). (After Sumner in *Journal of Genetics*.)

plete the analysis of the variable F_2 population. Later a difference of this sort was shown by Hoshino to be due to two independent genes. In other plants, estimates of the number of such gene differences between geographic races, differing in flowering time, range from 1 to 15 or more. Thus, geographic separation of populations appears to be often followed by an accumulation of many small gene differences.

This was first proved for animal populations by the careful work of Sumner, who collected many geographic races (subspecies) of deer mice (*Peromyscus*), which showed constant differences in coat color and other quantitative characters, and bred parent types, F_1 , and F_2 under observation in the laboratory. The results, of which Fig. 46 is an example, were similar for many characters. F_1 was intermediate between the parents; F_2 was intermediate and more variable, with some of the types within the range

of the parent types reappearing. Sumner, who at first believed the differences could not be explained by Mendel's principles, eventually showed that the multiple-factor theory provided the basis for explaining hereditary geographic variations.

The results of crossing members of different species point also in the same direction; multiple factors with small quantitative effects are certainly involved in some species differences, but the situation is so complex in respect to the large number of factors, the degree of sterility, and interference with the normal mechanism of segregation and assortment by abnormalities in chromosome pairing that it has defied detailed genetic analysis. Mather, who refers to quantitative characters controlled by many genes as *polygenic*, believes that these are the characters upon which natural selection acts by preserving those polygenic combinations which are best adapted to each other and to the environment.

The Number of Gene Differences in Quantitative Inheritance. By making the simple assumptions which East first used to permit comparisons between the general theory and the experimental results, namely, that the individual alleles in a series of multiple independent factors show no dominance and have about equal effects, which accumulate additively, it is possible to estimate the numbers of such gene differences between two races or strains which differ in a quantitative trait. The method depends on the assumption that the greater range and variability of F_2 over F_1 is a measure of the number of such gene differences between the parent races. This depends on methods for calculating the variabilities of the two populations and will be discussed in connection with biometric methods (p. 145).

It is important to realize that the number of multiple gene differences, when many are involved, can hardly be determined directly in the usual experimental populations. The reason is that the effects of one gene often interfere with or obscure or otherwise change the expression of another. In other words, the results are complicated by gene interaction, all the more so when the genes under observation affect the same character. Thus, in the pied mice described above, s produces almost no spotting effect in the combination $SsS_2S_2S_3S_3 \dots$, while it produces a good deal in the combination $Sss_2s_2s_3s_3 \dots$. This could be described by saying that $s_2s_2s_3s_3 \dots$ determine the dominance of S over s ; but this is merely to make a special case of the more general rule of gene interaction. Where interaction occurs, the assumptions of equal additive effect are unjustified and the number of genes can be estimated only by isolating them from the complex, one at a time, an operation so laborious that it is seldom undertaken. A partial analysis in the above case indicated that except for s , which has a fairly large effect, each of the other spotting genes (which are estimated to number from 4 to 10) has individually a very small effect. As more and

more of these are combined with *ss*, the coat becomes whiter, although when *SS* is substituted for *ss*, the other spotting genes are much less effective either separately or together.

Modifying Factors. The type of interaction, in which several genes with small expression, like s_2s_2 , s_3s_3 above, exert their chief effects by changing the magnitude of effect of a major gene, like *ss* above, is sometimes referred to as *modifying effect* and the minor genes as *modifiers*. In this case the modifiers have a small effect even when the major gene is not present, so that the effects of modifier and major gene cumulate. An analysis of genes producing white spotting in the guinea pig by Wright and Chase indicates a similar situation there, though probably with many more genes concerned. Castle, by selecting lighter and darker variants of the hooded (white-spotted) rat, produced strains which apparently differed by many modifiers with small effects, and this type of interaction is probably a common one. The complex of modifiers in a stock, which determines small effects and alters the expression of other genes introduced into it, is sometimes referred to collectively as the *residual heredity* of the stock. Because of the number of genes involved, it is usually difficult to analyze such complexes into their components.

In a few cases it has been shown that multiple genes, or polygenes, exist which have little or no effect themselves except in the presence of some specific main gene which they modify. Thus, in certain strains of mice, there occurs a form of spotting known as variegated (Fig. 47). Such variegated mice are heterozygous for a dominant gene *W*, which is lethal when homozygous, *WW* mice usually dying shortly after birth with a severe anemia. When, rarely, they live long enough to develop hair, they are seen to be entirely white with black eyes, that is, the white spotting covers the entire coat. The degree of white spotting of the heterozygotes, however, depends not only on *W* but on a number of multiple genes, each with a small effect, which determine the effect of *W* (Table XVII). If many of these modifiers are present, the *Ww* coat is nearly all white; if a few or none are present, *Ww* mice have little or no white. These modifiers have little or no effect in *ww* mice; even when the maximum number are present, the animal is unspotted unless *W* is also present. One effect of these modifying genes is thus to determine the degree of expression or dominance of *W*. When all of the modifiers are present, the cross of *Ww* × *Ww* produces offspring in the ratio of $\frac{3}{4}$ white ($\frac{1}{4}$ *WW*, $\frac{2}{4}$ *Ww*): $\frac{1}{4}$ unspotted (*ww*); when part of them are present, the offspring consist of $\frac{1}{4}$ white (*WW*), $\frac{1}{2}$ variegated (*Ww*), and $\frac{1}{4}$ unspotted (*ww*); and when none are present, the progeny are $\frac{1}{4}$ white (*WW*) and $\frac{3}{4}$ unspotted (*Ww* and *ww*). This is a clear-cut demonstration of the fact that dominance in one pair of alleles is determined by the interaction of other genes.

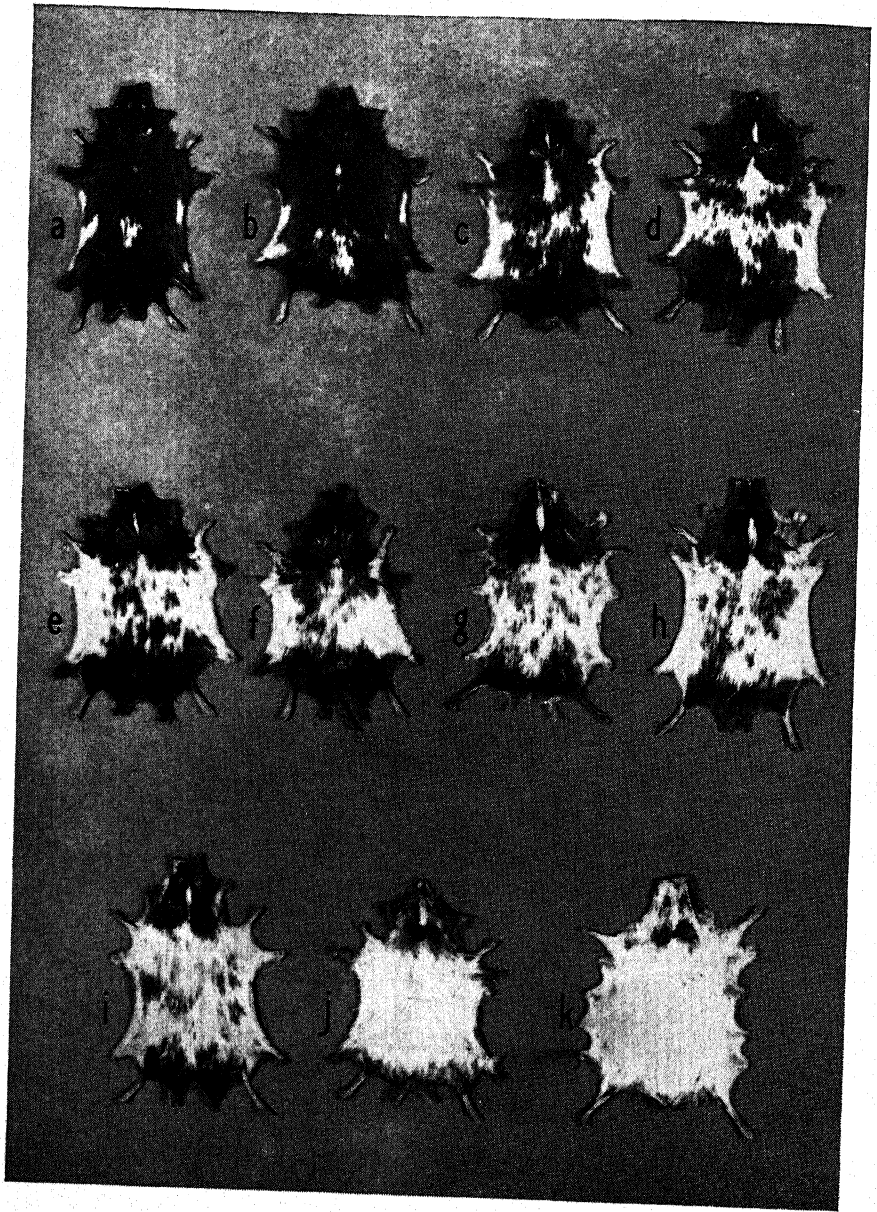


FIG. 47. Variation in the variegated white spotting in laboratory strains of mice differing in the number of mutant modifiers of the heterozygous effect of the gene W (in $WwSS$) from few (upper left) to many (lower right).

Modifiers like these may be referred to as *specific modifiers*, since their expression seems to depend upon interaction with one specific genotype. This type of interaction probably intergrades imperceptibly with the more general, less specific modifying effects of other genes with quantitative effects. Because of the mutual interaction among many genes in producing a phenotype it is probable that most genes may function as modifiers. Thus the gene yellow in the mouse, which has effects on coat color and viability, may also be treated as a modifier of spotting (cf. p. 144) and of

TABLE XVII. THE EFFECTS OF MULTIPLE SPECIFIC MODIFIERS $m(W)$ ON THE RELATIVE DOMINANCE OF THE SPOTTING MUTATION W IN HOUSE MICE

Parents	Progeny			
	Genotype for W and s	Condition of $m(W)$ loci	Phenotype in per cent dorsal white	Proportions
Light variegated $WwSS \times WwSS$	$WWSS$ $WwSS$ $wwSS$	$m_1 m_2 \dots m_x$	100 90-95 0-5	$\frac{3}{4}$ white $\frac{1}{4}$ unspotted
Unspotted $WwSS \times WwSS$	$WWSS$ $WwSS$ $wwSS$	$M_1 M_2 \dots M_x$	100 0 0	$\frac{1}{4}$ white $\frac{3}{4}$ unspotted
Medium variegated $WwSS \times WwSS$	$WWSS$ $WwSS$ $wwSS$	$M_1 m_2 M_3 m_x$	100 20-80 0	$\frac{1}{4}$ white $\frac{1}{2}$ spotted $\frac{1}{4}$ unspotted
Black-eyed-white $Wwss \times Wwss$	$WWss$ $Wwss$ $wwss$	$m_1 m_2 \dots m_x$	100 100 50-90	$\frac{3}{4}$ white $\frac{1}{4}$ spotted
Intermediate spotted $Wwss \times Wwss$	$WWss$ $Wwss$ $wwss$	$M_1 M_2 \dots M_x$	100 50-100 0-50	$\frac{1}{4}$ white $\frac{3}{4}$ spotted

size, since yellow mice have less white spotting and are generally larger than their nonyellow sibs. Other "color genes" in the mouse, as Castle has shown, also function as "size genes," some tending to increase, others to decrease the body weight.

Biometric Methods. Statistical methods have wide usefulness and importance in genetics, since the data of genetics are primarily derived from counting and measuring. A few methods for estimating errors of sampling in connection with Mendelian ratios have been given in previous chapters. Here we shall describe some techniques of computation by which the

measurements of transgressively varying characters may be reduced to simpler terms. Since the derivation and mathematical justification of these methods cannot be dealt with here, the student who intends to make extensive use of such methods should consult one of the standard treatises on biometry listed in the references.

The chief object of biometrical analysis as applied to quantitative characters is to reduce a mass of detailed data to some values or constants which succinctly characterize certain features of the population concerned. The statistical descriptions of different populations may thus be compared with each other or with ideal populations based on specific theories.

The biometric constants of greatest usefulness for such purposes are: the arithmetic average, or *mean*, which represents the magnitude of a given character in a population; the *standard deviation* and the *coefficient of variation*, which measure the variability of a population. In general, we can deal practically with *samples* of limited size and from these we may calculate *statistics* such as the mean and the standard deviation. We must then inquire in each case how well they represent the *population* from which the sample is drawn. Thus a parameter or *constant* refers to the population, a statistic to the sample. In comparing these we require a measure of the degree to which the sample represents the population and of the risk of error in using the part to represent the whole. This measure is the *standard error*.

Most organisms are variable, being played upon by environmental factors and by hereditary variability. When one of their characters is carefully measured, the variability exhibits certain regular features which can be utilized for description and comparison. If, for example, we measure, with a planimeter, the areas of colored and white spaces on skins from 200 mice of an inbred piebald line and state each measure as the per cent (to the nearest whole per cent) of the total skin which is white, we get 200 figures varying from 3 to 48 per cent. This *range* could serve as a first description of this sample. But a much clearer picture of this character appears when we classify the individuals into a smaller number of *groups*. By placing together all individuals, or *variates*, in classes differing by 5 per cent we obtain the *frequency distribution* of Fig. 48. The first class contains the 3 individuals with 3 to 7 per cent of white, the next whiter one the 15 with 8 to 12 per cent, and so on. The *mid-point*, or *center*, of the first is 5 (since it contains the numbers 3, 4, 5, 6, and 7), of the second the mid-point is 10; and these serve as *class values*, to represent the whole class. This process of gathering together similar variates is known as *grouping*.

The Frequency Histogram and Polygon. When the observations have been classified, the frequencies may be graphically represented by con-

structing a histogram as in Fig. 48, in which the segments along the abscissae represent the classes and the columns erected upon each segment are proportional in height to the number of individuals in that class. By

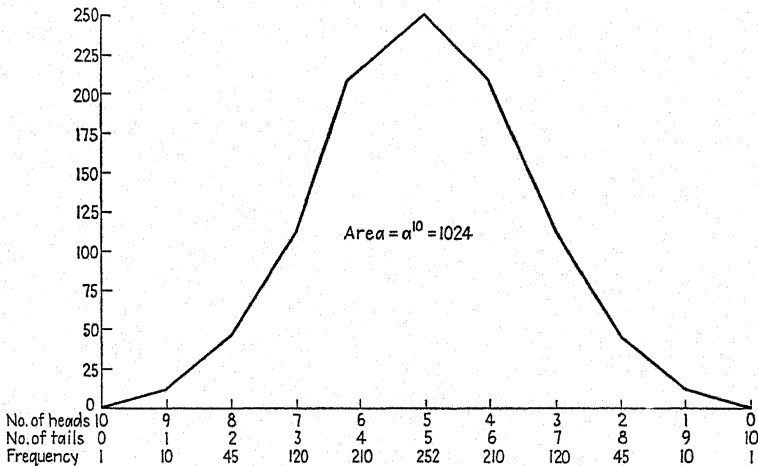
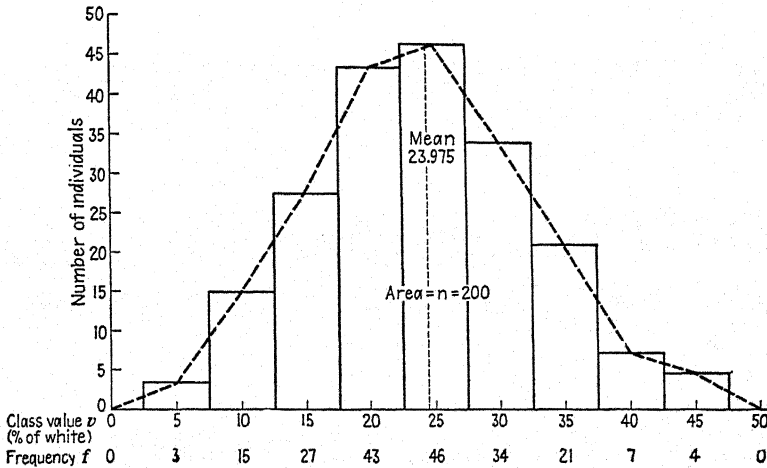


FIG. 48. Upper. Frequency polygon and histogram showing variation in extent of white areas in the skins of 200 pied mice.

FIG. 49. Lower. Frequency distribution and polygon showing the probabilities of coincidence of the given numbers of heads and tails in tossing ten coins $(a + b)^{10}$.

connecting the mid-points of each class, the frequency polygon is obtained. The area enclosed by the frequency polygon is proportional to the sum of all the frequencies, that is, the total number, n , of all of the individuals in the population.

Now the nature of the variation in white spotting becomes much clearer. The individuals with much and with little white are much less frequent than those with intermediate amounts, which constitute the great bulk of the population. Moreover, the variation is rather symmetrical about the largest class, or *mode*, of the distribution in the 25 per cent class, the frequencies falling off rather evenly toward each extreme.

The Normal Distribution. Such a distribution is found commonly when a group of individuals is classified according to any quantitative characters. It tends to approach what statisticians call the *normal curve* (Fig. 50), a curve representing the relative frequency with which, according to the laws of probability, various consequences may be expected to ensue from the simultaneous action of many independent causes. For example, if 10 coins were to be tossed simultaneously, the chances of all falling heads would be very slight and would be expected only once out of 2^{10} times, or once in 1,024 times. The chance of throwing 9 heads and 1 tail is somewhat greater; of 8 heads and 2 tails greater still; and the combination of 5 heads and 5 tails is the most likely of all. The chances of 2 heads and 8 tails, 1 head and 9 tails, and 10 tails are progressively less and less. The probability of each of these combinations corresponds to the coefficients of the various terms in the expansion of the binomial $(a + b)^{10}$, that is, $a^{10} + 10a^9b + 45a^8b^2 + \dots$. These probabilities are given in the frequency distribution in the lower part of Fig. 49 together with the graphical representation. The particular combination that appears in any given case is the result of 10 independent variables. The chance that they will all tend in the same direction is slight, and it is much more likely that some will tend one way and some another. Plotting the results of the interaction of these events that may be expected on the basis of pure chance produces a curve which approaches more and more closely to the normal curve as the number of factors increases. The normal curve, which is a theoretical construction, based on an infinite population, or having an area of 1, is a smooth, continuous curve of perfect symmetry and regularity, in which the mean, or average, bisects the population into two equal halves and coincides with the highest point, or *mode*. Many of the biometric methods used in dealing with populations showing continuous variation are derived from properties of the normal curve. Since it expresses the probabilities of coincidence of independent chance events [such as $(a + b)^n$], it is not perfectly exemplified by measurements of actual populations. Since, however, a group of individuals, classified for a given quantitative character, so commonly shows a curve like this, one is led to suspect that such characters are affected by a series of influences either in the environment or in the genetic constitution. In the present instance, we know that both genetic and nongenetic factors are concerned (p. 126). Some of these factors tend in one direction and some in the other, the character resulting from their

interaction usually finding its position at a point intermediate between the two extremes.

The division of a mass of data into classes and the plotting of these classes in a polygon or curve help in presenting a simple picture. However, the process does not provide two important descriptive expressions: a quantity that shall stand for the group as a whole and another that shall measure the variation within the group. These two constants must be derived from a study of the classified data.

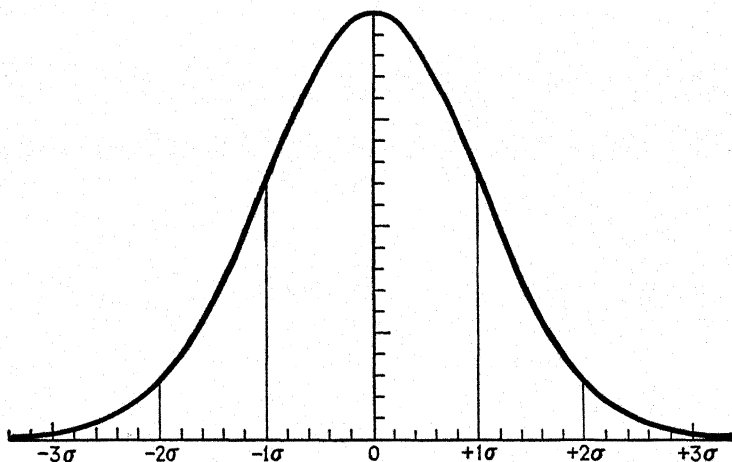


FIG. 50. A normal curve. Perpendiculars are erected above and below the mean at points once, twice, and three times the distance of the standard deviation from the mean.

The Mode. In a normal distribution there is usually one class, commonly situated about midway between the two extremes, which contains more individuals than do any of the others. This is known as the modal class, or the *mode*. For the group of mice studied, the class from 22 to 27 (Fig. 48) is the mode. Since the mode is the most populous of all the classes, it may be taken as a rough indication of the *type* of the population as a whole. If a single individual were to be selected as typical of the group, it would probably be chosen from among the members of the modal class. In the distributions found in actual samples, for example, in the measurements of height in a group composed of both girls and boys, there may be no single mode, or there may be two modes, one for girls and one for boys; or the mode may be nearer one extreme than the other so that the distribution is not symmetrical but may be skewed. In such cases the mode does not satisfactorily represent the group as a whole. The figure that best serves this purpose is the arithmetic average, or mean.

The Mean. One way of getting an average is to add together the values of all the individuals in a sample and to divide this by the total number.

This method of calculating the mean from the ungrouped frequencies is the most accurate but with large numbers may be very laborious. A simpler method and one nearly as accurate is to work from the grouped frequencies, as in Table XVIII, multiply the value of each class by the number in it, and divide by the total number. Some error is made in calculating from grouped frequencies since this assumes that the average of all individuals in the group is at the mid-point. The larger the class interval, the greater is this error likely to be. With fairly large samples little error is made if the class interval does not exceed about one-quarter of the standard deviation. If we denote the mean by \bar{m} and use V as value of class center, f as class frequency, n as total number of individuals, and Σ as summation symbol, then $\bar{m} = \Sigma fV/n$.

Thus, if we multiply each V by each f in the frequency distribution in Fig. 49, $(5 \times 3) + (10 \times 15)$, etc., we get $\Sigma fV = 4,795$. This, divided by 200, gives the mean = 23.975. This gives us a figure which does not represent any actual individual in the sample but stands for a central tendency of the group as a whole. The degree to which the mean represents the whole population will, of course, depend upon the dispersion or degree of variability of the population. If all individuals had exactly the mean value, the mean would represent them perfectly, but as the individuals are more and more scattered or dispersed, so that any one chosen at random is less and less likely to have an average, or mean, measurement, the mean provides a less and less accurate or reliable description of the group. Consequently it is necessary to have some measure of the variability of the population, not only to provide a measure of the reliability of the mean, but to disclose other characteristics of the population.

Standard Deviation. Populations which differ in variability are those in which the individual variates are differently dispersed about the mean. Thus the normal curves for two populations shown in Fig. 51 contain the same number of individuals (hence the curves enclose the same areas) and have the same mean; but in one the individuals are clustered closely about the mean, while in the other they are more widely dispersed. If in each figure we draw a pair of lines, Q_1 and Q_3 , each dividing the area on one side of the mean into equal parts, then the whole distribution will be divided into fourths, or quartiles. Half of the individuals will thus fall between the lines and half will fall outside; or, what amounts to the same thing, the chance is 50:50, or $\frac{1}{2}$, that any individual chosen at random will be drawn from the central group between the Q lines. Noting that the distance of the Q lines from the mean is quite different in the two curves, it can be seen that this distance could be used as a measure to compare the dispersion or variabilities of the two populations. Thus the area within which half the individuals in population *a* are found will be enclosed between the quartile

lines at the mean plus Qa_2 and mean minus Qa_1 , or $\bar{m} \pm Qa$; in population b , the same area will lie between $\bar{m} \pm Qb$. Since the value of Q is obviously greater in population b , we may say that its variability is greater than that of population a by the amount by which Qb exceeds Qa . These quartile values were formerly used as a measure of variability but in general have now been superseded by another criterion known as the standard deviation σ , which defines the distance on either side of the mean within which about two-thirds (actually 68.26 per cent) of the variates lie. It is thus a larger value than the quartile ($\sigma = Q/.6745$, or $Q = \sigma \times .6745$), is readily calculated from a frequency distribution, and has other properties which make it the most useful measure of dispersion. The standard deviation in any actual sample is thus a pair of values above and below the mean of a sample such that .6745 of the sample lies in the interval $m \pm \sigma$. In the diagram of a normal curve and in Table IV, p., 49 the proportions of the areas

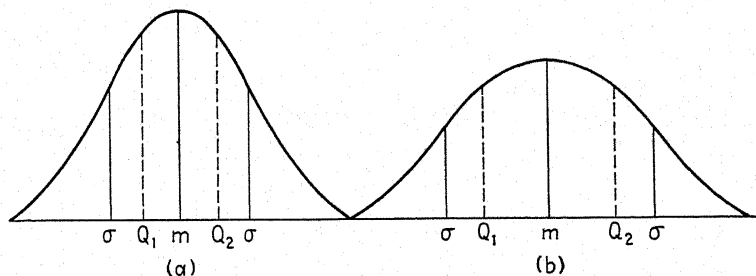


FIG. 51. Differences in variability of two populations shown graphically, (a) population of low variability relative to (b).

lying within and outside of the ordinates at various multiples of the standard deviation are shown. In all normal curves about 95 per cent of the area is within $\bar{m} \pm 2\sigma$, and over 99 per cent of the area is included within $\bar{m} \pm 3\sigma$. That enables us to say that, if we choose an individual at random from a normally distributed population, the chances are about 95 in 100 that it will belong to the part of the population included between $\bar{m} + 2\sigma$ and $\bar{m} - 2\sigma$ or that the chances are less than 1 in 100 that it will lie outside the limits of $\bar{m} \pm 3\sigma$. Thus the value of σ is a good description of the variability or dispersion of the population, a high value indicating a dispersed or variable population as in Fig. 51b, a lower value indicating a less variable, more compact population as in Fig. 51a.

Calculation of Variance and Standard Deviation. In practice we compute the deviation of each member of the sample (known as a variate) from the mean, square each deviation, add all the squared deviations, and divide by n . This gives us the mean square deviation, or *variance*. The standard deviation is the square root of the variance.

Computing the squared deviation of every variate in a large population would be a very laborious process, and so a short method has been developed by which both variance and standard deviation can be calculated at once from grouped data. This method, briefly, is to *assume* that the mean falls on an even class value, to measure the deviation from this assumed mean, a , and to make the necessary corrections at the end of the calculation. The mean may be assumed at a class which by inspection can be seen to be rather close to the true mean. If this is done, the deviations will be relatively small but some will be plus and some minus. It is a common practice to assume the mean at the smallest class or at zero, so that all the deviations are plus in sign. These deviations should be treated as units regardless of the actual class intervals and the calculations made on the basis of these units, thus avoiding the necessity for large numbers if the class interval is more than 1. The proper correction can be made by multiplying at the proper time by the class interval i , which will bring the results back to the unit of measurement employed.

In Table XVIII, the data on the frequency distribution in Fig. 49 have been arranged for calculation, and at the left the mean has been calculated as 23.975. In the fourth column, the mean is assumed to fall at the class center 25, and the deviations d are taken as whole classes, letting 5 per cent, the class interval, be represented by one. The sum of the products $f \times d$ (Σfd) is -41, which means that the true mean is less than the assumed mean by $-41/200$, or .205 classes. This is the correction factor. Since a class is 5 units, we multiply the correction factor by 5 and subtract the product from the assumed mean. This gives us the same mean as the calculation by the longer method. The formula for the mean by the short method is thus:

$$\bar{m} = a - i \left(\frac{\Sigma fd}{n} \right)$$

This method saves much labor in calculating the standard deviation, for the squared deviations of classes are much smaller numbers than the actual class values. The sum of the squared deviations is divided by n to obtain the variance, but since this is based on deviations from the assumed mean, the squared correction factor, $\Sigma fd/n$ must be subtracted from it. The square root of the remainder gives us the standard deviation in classes, so this must be multiplied by the class interval, 5, to give us the standard deviation in terms of the units of measurement used. The formula for the standard deviation by the short method is thus:

$$\sigma = i \sqrt{\frac{\Sigma fd^2}{n} - \left(\frac{fd}{n} \right)^2}$$

In the example, this is

$$5 \sqrt{\frac{579}{200}} - (.205)^2 = 8.45$$

At the end of this calculation we are able to say that the mean per cent of white in this strain of mice is 23.975 with a standard deviation of 8.45 per cent, which is to say that about two-thirds of these animals have amounts of white within 23.975 ± 8.45 per cent.

The Coefficient of Variation. The standard deviation is, of course, always in terms of the units used (per cent in our example), and its usefulness lies in comparing the variability of groups of individuals with regard to the same character. It is often necessary, however, to compare variability in one character with variability in another, for example, the variation in the weight of the men studied with the variation in their height; and for that purpose the standard deviation is useless, since pounds and inches cannot be compared. To avoid this difficulty, the *coefficient of variation*, v , is employed, which is merely the standard deviation divided by the mean and expressed as a percentage, its formula being $v = (\sigma \times 100)/\bar{m}$, which in the sample is $845/23.975$, or 35.2 per cent.

Measures of Reliability. The group of individuals from which a given statistic is derived (the 200 mice here studied, for example) are obviously only a small sample drawn from the entire population of which they form a part. How reliable a statistic is will evidently depend in part on how big the sample is. A mean weight derived from a sample consisting of only 10 men will command much less confidence than one derived from a sample consisting of 1,000 men, for the bigger the sample, the more accurately will it represent the entire population. But this is not the only criterion of reliability. If two samples are of the same size but differ in variability, more confidence is to be placed in constants derived from the less variable sample since in such a group it takes relatively fewer individuals to establish the character of the whole.

The Standard Error. These two qualities, therefore, the size and the variability of the sample or group studied, may be used to provide a measure of the reliability of constants derived from it. Such a measure is the *standard error*, an understanding of which requires a brief discussion of probability and of the properties of the normal curve.

If a series of samples were to be drawn from the same population as the one studied, the constants derived from them would probably not be the same. Another group of 200 spotted mice from the same stock would doubtless give a mean somewhat different from 23.975 per cent. If the differences were due entirely to chance and the samples were entirely random ones, it is a fair assumption from our knowledge of probability that

few samples would be relatively low and few relatively high and that most would be intermediate in value. Furthermore, if a large group of these sample means based on samples of the same size were plotted, they would

TABLE XVIII. CALCULATION OF MEAN AND STANDARD DEVIATION BY A SHORT METHOD, APPLIED TO DATA ON PER CENT OF WHITE SPOTTING IN EACH OF 200 MICE

V	f	Vf	d	fd	fd^2
5	3	15	-4	-12	48
10	15	150	-3	-45	135
15	27	405	-2	-54	108
20	43	860	-1	-43	43
25	46	1,150	0	0	0
30	34	1,020	+1	34	34
35	21	735	+2	42	84
40	7	280	+3	21	63
45	4	180	+4	16	64
$n = 200$		$\Sigma Vf = 4,795$		$\Sigma fd = -41$	$\Sigma fd^2 = 579$

Assumed mean, $a = 25$

Class interval, $i = 5$

$$\bar{m} = \frac{\Sigma Vf}{n} = \frac{4,795}{200} = 23.975, \text{ or}$$

$$\frac{\Sigma fd}{n} \times i = -.205 \times 5 = -1.025$$

Assumed mean = 25.

Correction = $\frac{-1.025}{23.975}$

$$\bar{m} = 23.975$$

$$\frac{\Sigma fd^2}{n} = \frac{579}{200} = 2.8950$$

$$\sqrt{2.8530} = 1.69$$

$$\times i \quad \frac{5}{8.45}$$

$$-\left(\frac{\Sigma fd}{n}\right)^2 = -(.205)^2 - \frac{.0420}{2.8530}$$

$$v = \frac{8.45 \times 100}{23.975} = 35.2\%$$

be again found to form a normal curve. The standard error is *the standard deviation of such a curve*. Since actually only one sample is at hand, this curve cannot be constructed and analyzed. From the size and variability of this single sample, however, the standard deviation of a group of con-

stants similarly derived may be calculated, since the more variable the sample, in relation to its size, the more variable might statistics be expected to be which were derived from similar samples.

The formulas used to derive the standard error of the mean, standard deviation, and coefficient of variation are as follows:

Standard error of the mean,

$$\sigma_{\bar{m}} = \frac{\sigma}{\sqrt{n}}$$

In our example $\sigma_{\bar{m}}$ is thus $8.45/\sqrt{200} = \pm .60$ per cent.

Standard error of the standard deviation,

$$\sigma_{\sigma} = \frac{\sigma}{\sqrt{2n}}$$

In our example σ_{σ} is thus $8.45/\sqrt{400} = \pm .41$ per cent.

Standard error of the coefficient of variation,

$$\sigma_v = \frac{v}{\sqrt{2n}} \sqrt{1 + 2\left(\frac{v}{100}\right)^2}$$

In our example σ_v is thus

$$\frac{35.2}{\sqrt{400}} \sqrt{1 + 2(.352)^2} = \pm 1.97$$

Where v is small, 10 per cent or less, the quantity under the second radical may be omitted. In our example this approximation is $\sigma_v = 1.76\%$.

In interpreting the meanings of these measures of reliability, we must again recall the properties of the normal curve (Fig. 50), especially the proportions of the total area included within $\bar{m} \pm \sigma$, $\bar{m} \pm 2\sigma$. If the value of a standard error of a constant is known, and this is simply the standard deviation of a population of constants similarly derived, then we have a criterion of the reliability of this constant. Since 68.26 per cent of the area of a normal curve and thus of a population distributed purely by chance may be expected to fall within $\bar{m} \pm \sigma$, we know the limits within which a constant derived from another sample may be expected to fall 68.26 per cent of the time. Thus when the mean per cent of white in the group of mice is $23.97 \pm .60$, this tells us that another sample from the same population would have, about two times out of three, a mean between 24.57 ($23.97 + .60$) and 23.37 ($23.97 - .60$). The standard error of the other constants has the same meaning: evidently the higher its standard error is, the less reliable is the constant. The standard error, as a criterion of reliability, is thus an important adjunct to any biometric expression and should always accompany each constant which is used.

Standard Error of a Difference. It is frequently necessary to test whether the differences between means or variabilities of two populations are real or whether they are merely due to chance differences in two different samples from the same population. For example, in crosses of yellow spotted mice by black spotted mice the amount of white spotting in yellow and black offspring was measured with the following results:

	<i>n</i>	\bar{m}	σ
Yellow.....	147	61.85 \pm 1.0	13.35 \pm .78
Black	137	72.05 \pm .96	11.25 \pm .68

The black mice have a higher mean and a lower standard deviation than the yellow ones. What is the likelihood that this is due to chance, that is, that the differences in the means and standard deviations reflect, not real differences between blacks and yellows, but only accidents of sampling? In order to answer this question we must know how to calculate the standard errors of the differences between the constants. This rests on the rule that the standard error of a difference of independent variables is the square root of the sum of the squares of the standard errors of the two constants being compared, that is:

$$\sigma\Delta_{1,2} = \sqrt{\sigma_1^2 + \sigma_2^2}$$

In this case

$$72.05 \pm 1.10^2 = 1.21$$

$$61.85 \pm .96^2 = .9216$$

$$10.20 \pm \sqrt{1.21 + .9216} = 1.46$$

The difference is 7.0 times as great as its standard error, that is, 10.20/1.46 = 7.0, and by consulting an extension of Table IV, page 49 we find that such a difference of 7.0, if due to chance, would be found only once in over 17,000 such comparisons; that is, it has a probability of only about .000058, hence is almost certainly not due to chance.

The difference in variability between yellows and blacks is calculated below:

Yellow	13.35 \pm	(.78 ²)	=	.6084
Black	11.25 \pm	(.68 ²)	=	.4624

$$\text{Difference} \quad 2.10 \pm \sqrt{1.0708} = 1.03$$

This is probably a real difference since it is twice as large as its standard error ($2.10/1.03 = 2.04$), but since a difference of this kind might arise by chance in 4.5 per cent ($2\sigma = .0456$, Table IV, p. 149) of similar comparisons, we cannot be so confident of this difference as in the case of the means. The conclusion derived from these calculations was that since yellow mice showed significantly less white spotting than nonyellow ones with the same spotting genes, the yellow mutation probably acts as a modifier of spotting. This was confirmed by other experiments.

The Significance of Deviations from Expectation. Methods for calculating the likelihood of given departures from an expected ratio by means of standard errors and the χ^2 test have already been described (pp. 48 and 70). The χ^2 test is also useful in comparing actual frequency distributions such as F_2 populations segregating for multiple factors, with expected ones, such as those arising from assumptions concerning the number and interaction of a set of polygenes. It may also be used to determine the likelihood that two actual distributions, such as two F_2 's, have been drawn from the same population. For these and many similar uses, a treatise on biometric methods should be consulted.

Estimating Number of Gene Differences. The methods for calculating variance may now be used to get some idea of the number of genes concerned with a difference in a quantitative character in two populations. Since the F_1 is usually intermediate and no more variable than the parent populations, while F_2 is usually more variable than F_1 because of segregation, it is theoretically possible to estimate how many genes are segregating by measuring the relative increase of variance of F_2 over F_1 . Consider the four F_2 populations in Table XIX. Assume that each population is derived from crosses between populations one of which is homozygous for small size, 1, and the other for large size, 13, and that all the difference is due to genic causes. If one pair of genes without dominance causes the difference between class 1 and class 13, segregation occurs in a 1:2:1 ratio and the variability of the population is high, $\sqrt{18}$. If the same difference

TABLE XIX. THEORETICAL FREQUENCY DISTRIBUTION OF A QUANTITATIVE CHARACTER IN FOUR F_2 POPULATIONS IN WHICH THE DIFFERENCE BETWEEN THE EXTREME TYPES DEPENDS ON ONE, TWO, THREE, AND SIX PAIRS OF INDEPENDENT GENES

Gene pairs	Class centers													<i>n</i>	σ
	1	2	3	4	5	6	7	8	9	10	11	12	13		
1	1	2	1	4	$\sqrt{18}$
2	1	4	6	4	1	16	$\sqrt{9}$
3	1	...	6	...	15	...	20	...	15	...	6	...	1	64	$\sqrt{6}$
6	1	12	66	220	495	792	924	792	495	220	66	12	1	4,096	$\sqrt{3}$

is due to two independent genes, all with equal additive effect ($A = a = B = b$), the standard deviation is lower, $\sqrt{9}$. As the number of genes acting in this way increases, the variability of the F_2 distribution decreases. If the variability of F_1 is the same in each case, it follows that the difference in variance between F_2 and F_1 is a function of the number of genes involved. An approximate estimate is given by $n = D^2/8(\sigma_{F_1}^2 - \sigma_{F_2}^2)$, where D is the difference between the parents' means of $D = \bar{m}_2 - \bar{m}_1$. The assumptions of equal additive effects of genes, none of which are linked with any others, and of homozygosity of all genes in one parent stock and of the alleles of these in the other will be recognized as rather artificial and not likely to be justified in any actual case. Since departures from the assumptions, such as dominance, lead to underestimates of n , the formula may be used to get minimum estimates. For example, Goodwin has by this means estimated that a minimum of nine genes are concerned in the difference in flowering time between geographic races of goldenrod from the northern and southern parts of the United States. Even though such genes probably cannot be isolated and studied separately, a consideration of the factors responsible for the differences in variance of quantitative characters in F_1 and F_2 is often a useful starting point for an understanding of the polygenic inheritance which plays so important a part in differentiating races and species.

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PROBLEMS

175. The F_1 generation from pure parent types differing in a size character is usually no more variable than the parents. Explain.

176. If two pure types, differing in a size character, are crossed, is it possible for individuals in the F_2 to be more extreme than either grandparent? Explain.

177. Why is it, when selection has ceased to be effective in producing changes in a given stock, that if this stock is crossed with another similar one selection among the subsequent offspring is often able to produce a marked change?

178. As a result of crosses involving a size character, it is often found that F_3 families raised from selfed F_2 plants differ markedly in their variability. Some are almost as low as the original parents, some a little higher, and some as high as the F_2 itself. None exceeds the F_2 in variability, however. Explain these facts.

179. It frequently happens that one character of a plant, such as number of seeds, is much more variable than another character, such as weight of seeds. What explanations for this difference can you suggest?

180. Certain groups of individuals, when their frequency distribution is plotted, show a bimodal or multimodal curve. What different explanations can you make for this fact?

Note. Assume that in man the difference in skin color between Negro and white is due to two pairs of factors; that $AA BB$ is "black" and $aa bb$ "white"; and that any three of these factors produce "dark" skin; any two, "medium"; and any one, "light."

181. What will be the skin color of the offspring from a mating of white with black? from a mating of two individuals genotypically like these F_1 offspring?

182. What are the genotypes of the parents in the two following matings of Negroes: medium \times light, giving one-eighth dark, three-eighths medium, three-eighths light, one-eighth white; medium \times light, giving one-half medium and one-half light?

183. Can two Negroes have white-skinned offspring? Can two white-skinned people have dark-skinned offspring? Explain.

184. Assume that the red kernel color of a certain race of wheat is due to the presence of three independent genes R_1 , R_2 , and R_3 . Any one of the genes singly will cause the red color. White is $r_1 r_1 r_2 r_3 r_3$. What are the genotypes of the parents in each of the following crosses: red \times red giving 3 red:1 white; red \times red giving 15 red:1 white; red \times red giving 63 red:1 white; red \times red giving 7 red:1 white; red \times white giving 1 red:1 white; red \times white giving 3 red:1 white; red \times white giving 7 red:1 white; red \times white giving all red?

185. Assume that the difference between a race of oats yielding about 4 grams per plant and one yielding 10 is due to three equal and cumulative multiple-factor pairs $AA BB CC$. Cross one type with the other. What will be the phenotypes of the F_1 ? of the F_2 ?

186. Assume that in squashes the difference in fruit weight between a 3-pound type and a 6-pound type is due to three factor pairs AA , BB , and CC , each factor contributing $\frac{1}{2}$ pound to fruit weight. Cross a 3-pound plant ($aa bb cc$) with a 6-pound one. What will be the phenotypes of the F_1 ? of the F_2 ?

187. In the following squash crosses, what will be the range in fruit weight of the offspring, on the previous assumption:

$$\begin{array}{l} Aa Bb CC \times aa Bb Cc \\ Aa bb Cc \times Aa BB cc \end{array}$$

$$\begin{array}{l} Aa Bb Cc \times Aa Bb Cc \\ aa BB cc \times AA BB cc \end{array}$$

188. Assume in the following three problems that the difference between a corn plant 10 decimeters high and one 26 decimeters high is due (in so far as it is caused by inheritance) to four pairs of equal and cumulative multiple factors, the 26-decimeter plant being $AA BB CC DD$ and the 10-decimeter one $aa bb cc dd$. What will be the size and genotype of an F_1 from a cross between these two pure types? Give the limits of variation in height which the offspring of the following crosses will show:

$$\begin{array}{l} Aa BB cc dd \times Aa bb Cc dd \\ aa BB cc dd \times Aa Bb Cc dd \end{array}$$

$$\begin{array}{l} AA BB Cc DD \times aa BB cc Dd \\ Aa Bb Cc Dd \times Aa bb Cc Dd \end{array}$$

189. Two 14-decimeter corn plants, when crossed, give nothing but 14-decimeter offspring. Two other 14-decimeter plants give one 18-decimeter, four 16-decimeter, six 14-decimeter, four 12-decimeter, and one 10-decimeter plants. Two other 14-decimeter plants when crossed give one 16-decimeter, two 14-decimeter, and one 12-decimeter plants. What genotypes for each of these 14-decimeter parent plants would explain these results? By selection in any of these families would it be possible to get a plant taller than 18 decimeters?

190. A breeder has a 26-decimeter starchy and a 10-decimeter sweet corn. Starchiness is dominant over sweetness and is due to a single factor. He wants a 26-decimeter sweet corn. Assume that height is due to four factor pairs, as before. If he wants this new type of corn in two years, how many plants should he raise in the F_2 of the cross between tall, starchy and short, sweet to be reasonably sure of getting it? If he has more time, what would you advise him to do in order not to have to raise such a big crop in the F_2 and subsequent generations?

191. Calculate the means, standard deviations, and coefficients of variability,

and their standard errors, from the data on ear length in maize in Fig. 44. About how many gene differences concerned with ear length do you think there are between the parent varieties?

192. Calculate means, standard deviations, and coefficients of variability, and their standard errors, from the data on corolla length given in Table XX. Do you think that more or fewer gene differences are concerned in this cross or in the one in Problem 191.

TABLE XX. FREQUENCY DISTRIBUTION FOR COROLLA LENGTH IN A CROSS BETWEEN VARIETIES OF *Nicotiana longiflora* Cav. (From East)

	Class centers, millimeters																			
	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91
P ₁	1	21	140	49																
P ₁	13	45
F ₁	4	10	41	75	40	3							
F ₂	1	5	16	23	18	62	37	25	16	4	2	2		

193. Compare the standard deviations for amount of spotting of Line 118 and Line 190a, Table XVI, and calculate the standard error of the difference. Suggest explanations for the result.

CHAPTER VII

THE PHYSICAL BASIS OF INHERITANCE

As has been pointed out in the foregoing chapters, all life may be viewed as depending on an interaction of heredity and environment. Every structural, physiological, or psychological trait of a living individual is determined by the interactions between its genotype, and the succession of environments in which it develops. A great deal of new insight into the nature of heredity was gained from the work of Mendel and his immediate successors. By breeding experiments with genotypically different strains of plants or animals, it was possible to demonstrate that the inherited constitution consists of discrete units, the genes. In many ways, the gene theory of modern biology resembles the atomic and molecular theories of physics and chemistry. All these theories are *particulate*, or *corpuscular*, since they endeavor to describe the phenomena which they study in terms of the behavior of more or less independent and discrete units.

Formal Genetics. Nobody has ever seen, and possibly no one will ever see, genes, molecules, or atoms. This does not make us reject these corpuscular theories. Their justification lies in the fact that they make intelligible many relationships which would be otherwise confused and bewildering. Thus, all kinds of chemical reactions can be understood as resulting from interactions of less than a hundred different kinds of atoms. Similarly, the results of hybridization experiments with all kinds of plants and animals can be elucidated by analyzing them in terms of genes, as shown in Chapters II to VI. Furthermore, the atomic, molecular, and gene theories often permit us to predict the results of new, hitherto untried experiments. For example, a knowledge of the segregation in the F_2 generation of a given hybridization experiment often permits us to foresee correctly the situation which arises in test crosses, and vice versa. Now, when a scientific hypothesis not merely continues to explain satisfactorily all the facts brought forward by observation and experiment, but also serves as a reliable guide in predicting the outcomes of new experiments, it becomes accepted as an established scientific law. A *law* is thus a brief statement or explanation of some relationship which has been found to hold through a large series of natural events.

First formulated by Mendel in 1866 and rediscovered in 1900, the laws of gene transmission have been tested and retested by applying them to

all kinds of hybridization experiments, including some which seemed at first to be incompatible with the theory, for example, the inheritance of quantitative characters. For the purposes of analyzing the inheritance of traits in crosses it is sufficient to define a gene as a unit, transmitted from parents to offspring, which is responsible for the development of certain traits in individuals living in certain environments. The gene so defined is really a symbol or abstraction, and the body of knowledge concerned with this symbolic gene has come to be known as *formal genetics*. This development represented an enormous advance in understanding of the phenomena of heredity as compared with the pre-Mendelian period. Formal genetics was developed in essentially modern form during the first quarter of the twentieth century.

But the matter could not be allowed to rest there. Many investigators have endeavored to discover just where in the organism the genes are located, what they are, and how they function, in other words to discover the material, or physical, basis of heredity. The efforts of these investigators have been brilliantly successful, and it is now known that genes are particles borne in the chromosomes of cell nuclei. Although genes are too small to be seen with the aid of even the best existing microscopes, the behavior of the chromosomes can be studied visually, and such studies reveal many things about the genes. Thus genetics has joined forces with *cytology*, the science dealing with cell structures and cell functions. This union of genetics and cytology has become so intimate that in many studies it is impossible to delimit the concepts and theories of one science from those of the other; for such studies the hybrid name *cytogenetics* is sometimes used. In the nineteenth century the study of cells was, however, largely independent of the study of heredity, and thus the pioneers of cytogenetics in the early years of the present century found an appreciable body of knowledge ready to be interpreted.

The Cellular Basis of Reproduction. The first light of understanding of the processes of reproduction dawned in the seventeenth century, when Harvey in England studied the early animal embryo. Without the aid of a microscope, Harvey was, however, unable to discover mammalian egg cells and thought that mammalian embryos are somehow secreted by the wall of the uterus. The Dutch embryologist De Graaf was the first to find that egg cells of a rabbit originate in the ovary (1672), but the full story of the origin and fertilization of eggs in mammals was first unraveled in his studies of the dog by the great embryologist von Baer (1827). Meanwhile spermatozoa had been discovered by the pioneer microscopist Leeuwenhoek (1677). Within a century thereafter, Swammerdam (1737) succeeded in artificially inseminating frog's eggs, and Spallanzani (1785) obtained offspring from artificial insemination in dogs.

Reproductive phenomena in plants were clarified by Camerarius (1694), Kölreuter (1760), and Sprengel (1793). Thus, by the end of the eighteenth century it was clear that eggs and spermatozoa in animals and ovules and pollen in plants constitute the physical connection between parents and offspring. Heredity must consequently be transmitted through these germinal structures.

Further progress in understanding the physical basis of heredity became possible through the development of the cell theory by several early microscopists, which was finally established by Schleiden and Schwann (1838)

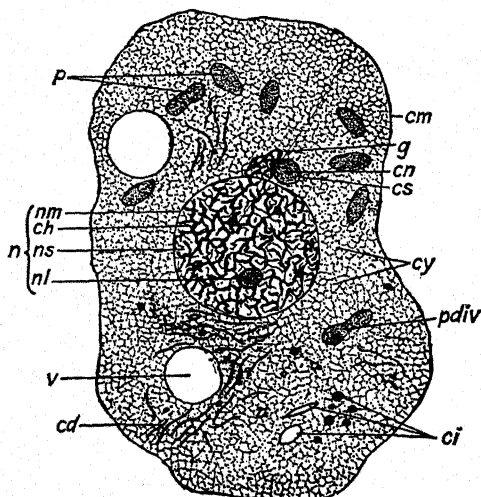


FIG. 52. A typical cell: *cd*, mitochondria; *ch*, chromatin; *cm*, cell membrane; *cn*, centriole; *cs*, centrosphere; *cy*, cytoplasm; *g*, Golgi apparatus; *n*, nucleus; *nl*, nucleolus; *nm*, nuclear membrane; *ns*, nuclear sap; *p*, plastids; *pdiv*, plastid dividing; *v*, vacuole. (From Shull, La Rue, and Ruthven.)

and Virchow (1855). According to this theory, all organisms are composed of one or of many living units called cells. Cells arise only from the division of preexisting cells. Germ cells come from division of certain cells in the body of the parent and in turn produce by division the cells of the body of the offspring. Thus, the human body starts from a single cell, the fertilized egg, but by the time of birth it consists of about 200 billion cells (2×10^8).

Most, and perhaps all, cells consist of a denser mass of protoplasm, the *nucleus*, surrounded by *cytoplasm* (Fig. 52). Many cells, especially in plants, are surrounded by a cell wall of cellulose or related substances, but other cells, including those of animals, lack a pronounced wall and are enclosed only in a semipermeable membrane. Cytoplasm contains a colloidal ground substance in the composition of which complex protein substances

play an important role. Cytoplasm may show also various structures and inclusions, such as centrosomes and centrioles, which perform important functions in cell division, mitochondria, Golgi bodies, and plastids, which are instrumental in synthetic and secretory activities of cells, and various vacuoles, fat globules, and starch grains, which are also important for food storage and in other ways. The cytoplasmic structures are often strikingly different in different cells of the same body, as well as in cells of different organisms.

The nuclei of cells are relatively more constant, at least in cells of the same species. A nucleus contains a certain number of bodies called *chromosomes* (Waldeyer 1888), which can be seen particularly clearly when the nucleus is in the process of dividing. In most organisms which reproduce sexually, chromosomes regularly occur in pairs, of which one member has come from the father, the other from the mother. The numbers of chromosomes vary in body cells from 3 pairs (in the plants *Crepis* and *Drosophyllum* and in some species of *Drosophila*) to more than 100 in some moths, crayfish, some plants, or certain Protozoa.¹ Man has 24 pairs of chromosomes in the body cells. The gametes contain half as many chromosomes as the body cells do; this is because the formation of gametes involves the process of chromosome reduction, or meiosis, described below (p. 160). Most nuclei also show, during the intervals between divisions, one or more bodies called *nucleoli*, which stain with certain dyes differently from the chromosomes. Nucleoli are formed by the chromosomes; they arise usually at fixed points of certain chromosomes, these points being accordingly called *nucleolus-forming* organs.

Cells of some lower organisms, especially bacteria, were for a long time believed to be devoid of nuclei. Recent investigations, particularly those of Robinow, make it probable that nucleuslike or chromosomelike bodies (Fig. 53) occur in at least some of these simple forms of life. The apparently still simpler forms, bacteriophages and viruses, show, significantly enough, a resemblance to chromosomal materials in chemical composition, the class of compounds known as nucleoproteins being the principal constituents in all these instances.² Viruses have, indeed, been compared to "naked genes."

Mitosis. Cell nuclei were first seen and named by Robert Brown in 1831, but their biological significance became apparent only when Stras-

¹ Some cells of a race of *Ascaris megalocephala*, a round worm parasitic in the intestine of horses, show only a single pair of large chromosomes. These chromosomes are, however, compound structures which break up in other cells of the same animal into numerous small chromosomes.

² Some of the viruses contain, however, so-called desoxyribose nucleic acids, which occur also in the chromosomes, while other viruses show the ribose nucleic acids, which occur in the cytoplasm as well as in the nucleolus of higher organisms.

burger (1875), Bütschli (1876), and others discovered that nuclei arise exclusively from other nuclei by means of a remarkable process of division which was called *mitosis* by Flemming (1882) (Fig. 54). Nuclei are, therefore, essential cell organs which cannot arise from cytoplasmic constituents.

During the period of *interkinesis*, which intervenes between successive nuclear and cell divisions, separate chromosomes are usually not distinguishable in the nucleus in microscopic preparations. The nuclear cavity shows instead chromatin granules and fibers stainable by certain dyes, surrounded by nuclear sap. Interkinesis has often been called resting stage, but this name is misleading since this stage of the life cycle of cells is probably the period of most lively chemical synthetic activity on the part of the genes.

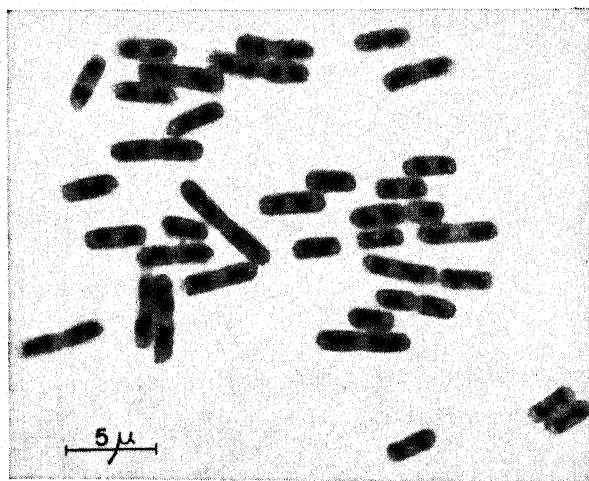


FIG. 53. Dividing nuclei in bacterial cells (*Bacillus cereus*). (After Robinow.)

When a cell is preparing to divide, the chromosomes become clearly visible as stainable threads which gradually shorten and thicken by means of coiling, or spiralization. This is the *prophase* stage. The better stainability of chromosomes at prophase and the following stages is due to accumulation of deoxyribose nucleic acid in the body of the chromosome.

The nuclear membrane disappears, and a spindle-shaped structure appears in which "spindle fibers" denser than the surrounding cytoplasm connect the chromosomes with the two poles of the nucleus. On animals these poles are marked by the *central bodies*, or *centrosomes*. The chromosomes now are arranged in a single plane about midway between the two poles of the spindle to form an *equatorial plate*. This stage of mitosis is known as the *metaphase*; chromosomes are most easily visible and countable during the metaphase.

At some time during the prophase or metaphase each chromosome be-

comes visibly split along its length into two daughter chromosomes. There is no agreement among cytologists as to just when the actual duplication of the chromosome, which must involve the production of a duplicate of every gene in the chromosome, takes place. Interkinesis is the most likely stage at which this synthetic activity may take place, but some authors believe that it happens during the preceding cell generation. If so, a meta-

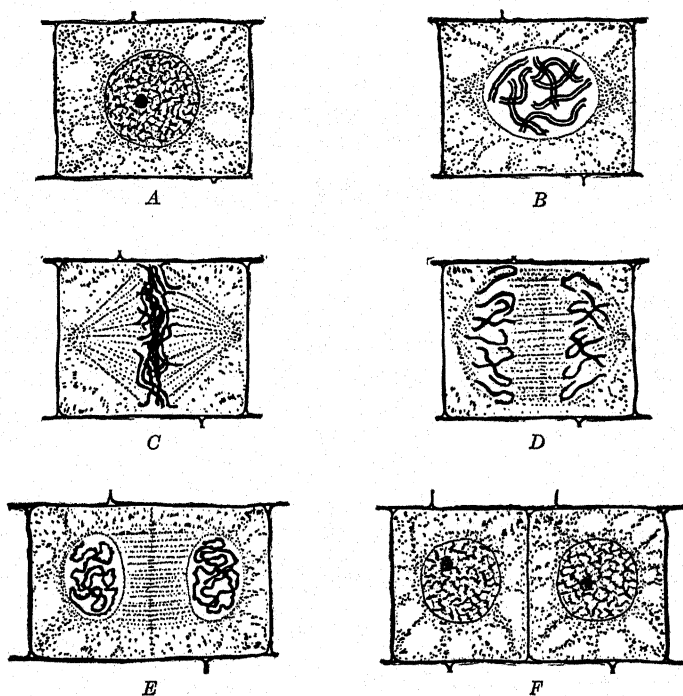


FIG. 54. Diagram of cell division by mitosis: *A*, resting cell, the chromatin of the nucleus in a fine network; *B*, *prophase*, the chromatin is gathered into long threadlike double chromosomes; *C*, *metaphase*, the split chromosomes arrange themselves in a plane across the equator of the cell, and the spindle, with its two poles, is formed; *D*, *anaphase*, the chromosome halves separate, one complete set (eight in this case) going to one pole and the other set to the other pole; *E*, *telophase*, each new group of chromosomes arranges itself into a thread, and a new cell wall begins to appear between the groups; *F*, two complete new cells, each with a nuclear content equal and similar to that of *A*.

phase chromosome is actually quadruple, rather than double. It is possible that the situation may not be identical in different cells and in different species. However that may be, at the end of metaphase the daughter halves of each chromosome begin to diverge from each other and eventually pass to the opposite poles of the mitotic spindle. This separation of the daughter chromosomes constitutes the anaphase of mitosis. The details of the anaphasic movements of chromosomes again differ in different organisms. In most cases, each chromosome contains at a fixed point of its body

a minute structure called the *centromere* which seems to be primarily concerned with the anaphase movements of the chromosomes and which leads the way on the spindle toward the poles, the rest of the body of the chromosome apparently following the centromere passively. In some insects, however, the centromeric activity is manifested at many points, or perhaps even along the entire length of the chromosome.

The anaphase merges into the *telophase*, during which the daughter chromosomes assembled at the poles of the spindle become included in a new nuclear membrane. The chromosomes gradually lengthen, uncoil, and become less darkly stainable, on account of a decrease in the amount of desoxyribose nucleic acid, which determines the staining properties of a chromosome. Meanwhile, the mitotic spindle disappears, and a new cell wall is laid down in the equatorial plane between the nuclei (in plants) or the cell is divided into two daughter cells by a cleavage furrow (in animals). The cycle is completed by the advent of a new *interkinesis*.

The discoverers of mitosis were profoundly impressed by the orderly complexity of this process. All the elaborate maneuvers which constitute mitosis result in qualitatively and quantitatively precise duplication and division of the contents of each chromosome of the mother nucleus between the nuclei of the daughter cells. Weismann (1892) and Roux (1905) concluded that no substances other than those concerned with the transmission of heredity could require so meticulously accurate a division and distribution. Accordingly, hereditary materials were supposed to be borne in cell nuclei and in their chromosomes. Similar views were expressed earlier also by Strasburger (1884) and O. Hertwig (1884) on the basis of studies on the processes of fertilization.

Fertilization or Syngamy. Sexual reproduction occurs in all kinds of organisms, from unicellular algae and protozoans up to the highest plants and animals. Lederberg has recently shown that Mendelian segregation and hence probably sexual fusion may take place also in some bacteria, and the work of Luria suggests that the same may be true even in bacteriophages. The sexual union is accomplished by a great variety of means in different organisms. The simplest method is probably *isogamy*, where the gametes that unite are similar in size and in structure. Isogamy occurs only in some of the lower organisms. Elsewhere, the gametes are more or less unlike (*anisogamy*, or *heterogamy*). Here there is a division of labor between the female and male gametes: the eggs are much larger than the sperm, and their abundant cytoplasm is laden with stored food materials which are used later by the developing embryo. Male gametes are usually motile; their entire organization is adapted to reach and to penetrate the egg. The amount of cytoplasm in male cells is reduced to a minimum. The only structures that are at all similar in female and male gametes of

the same species are their nuclei and particularly the chromosome complements contained in the nuclei.

The essential features of fertilization are the same wherever it occurs. This remarkable and significant uniformity was perceived by O. Hertwig in 1875 and Fol (1877) working with marine animals and by Strasburger (1877, 1884), who studied both algae and higher plants. In all cases the crucial event of fertilization is the *fusion* of the nuclei of male and female gametes to form a single zygotic nucleus. Each gamete contains a *haploid* set of chromosomes, that is, one member of each of the pairs of chromosomes which characterize the species. In man, for example, the haploid set is 24, in maize 10, in *Drosophila* 4. The zygote nucleus contains, of course, one set from each of the uniting gametes, always the sum of the two gametic sets. Thus the zygotic or diploid chromosome complement is 48 in man, 20 in maize, 8 in *Drosophila*, etc. These facts may also be expressed by saying that gametes have n chromosomes and zygotes $2n$.

Now, the traits of a child are influenced by the maternal and the paternal heredities to about the same extent. This means that the mother and the father, or the female and male gametes, are equally efficient in transmitting heredity. The pioneer students of fertilization asked themselves the following question: How can the female and male gametes, which are very different in anisogamic organisms, transmit the same heredity to the same extent? Unless one makes the improbable conjecture that similar hereditary endowments are carried in different structures in female and male gametes, the only solution is that both of these types of cells must have some structures that are similar and that it is these similar structures which are the principal carriers of heredity, while the remaining portions of the cells are subsidiary. As stated above, the parts that are similar in female and male gametes of the same species are the nuclei and particularly the chromosomes. These, accordingly, may be supposed to be the carriers of heredity.

That both female and male gametes may each contain all the hereditary factors which are necessary for the development of the organism can also be demonstrated by different methods. In some forms the eggs develop into a new individual without having been fertilized, a process called *parthenogenesis*. Parthenogenesis was discovered in plant lice (Aphidae) as early as 1745 by Bonnet; in these insects an alternation of parthenogenetic and sexual generations is a normal phenomenon. Perhaps even more important for our purpose is that parthenogenetic development of eggs may be induced artificially also in some species which normally reproduce exclusively by syngamy. The first instance of artificial parthenogenesis was obtained by Tichomirov (1886) in the silkworm moth, and soon several investigators had independently induced parthenogenesis in

the eggs of marine invertebrate animals. Even the eggs of certain vertebrates, for example the frog, can be caused to develop without fertilization. Therefore, the egg nucleus alone is sufficient for approximately normal

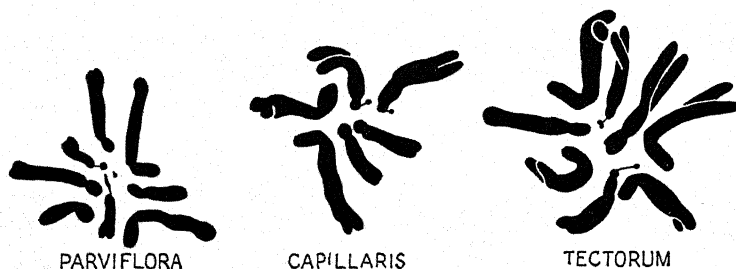


FIG. 55. Diploid chromosome complements of three species of *Crepis*, a plant of the family Compositae, showing chromosome individuality. (After Hollingshead.)

development. A similar demonstration of the sufficiency of sperm nuclei was given by experiments on *merogony*, in which the egg nucleus is first removed or destroyed without serious injury to the egg itself and then the

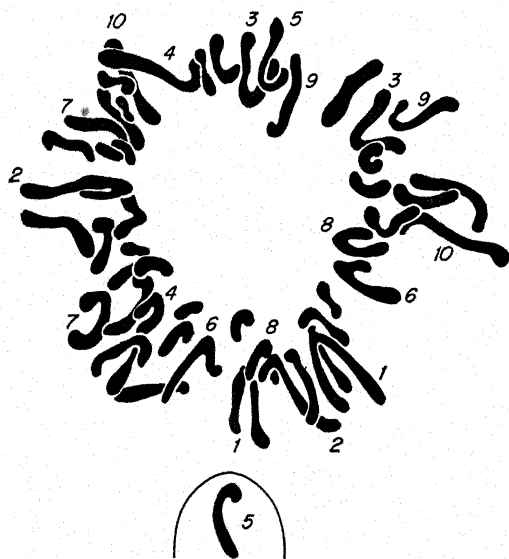


FIG. 56. Chromosomes of a fibroblast cell from tissue culture preparation from a human embryo. Note similarities of the members of some of the 24 pairs of chromosomes. (After Andres and Navaschin.)

enucleated egg is fertilized by normal spermatozoa. Such embryos develop under the direction of the sperm nuclei.

Individuality of Chromosomes. Thus far we have treated nuclei with their chromosome complements as units. This is just as they were treated

by the pioneer cytologists, who drew, nevertheless, the correct inference that nuclei and chromosomes are the carriers of heredity. The next step in the study of the physical basis of inheritance was the transfer of attention to separate chromosomes by van Beneden (1883) and especially by Boveri (1887) and later workers. It was found that the number of pairs of chromosomes is constant in all the individuals of the same species. Moreover, in many organisms, the different pairs of chromosomes are visibly different in size and in shape in the same nucleus (Fig. 55). For example the common weed *Crepis capillaris*, has three pairs of chromosomes: a pair of long rods with arms, divided by the centromere, of unequal length with a ratio of about 3:1; a pair of shorter chromosomes with a short arm to which a small stalked piece or satellite is attached; and a shorter pair with one arm very much shorter than the other. The three types of chromosomes are sharply distinguished by size and form.

Similarly, the diploid cells of *Drosophila melanogaster* contain the following four pairs of chromosomes: (1) a pair of large V-shaped chromosomes with median centromeres; (2) a pair of somewhat smaller V-shaped chromosomes with median centromeres and usually also with deep *secondary constrictions* in one arm of the V; (3)

a pair of rodlike chromosomes; and (4) a pair of short rods, or dotlike chromosomes (Fig. 69, p. 177). The haploid chromosome complement in the same species (found in maturing germ cells) contains four chromosomes, one of each of the four kinds just described. The paired condition in the diploid cells is due to one member of each pair coming from the mother and the other from the father. Diploid cells of the human body contain 24 pairs of chromosomes, and at least some of them are distinguishable by size and shape (Fig. 56). In the maize plant (Indian corn) each of the 10 pairs of chromosomes can be distinguished by the length, the location of the centromeres, and deeply staining knobs, which give each chromosome, as viewed at the pachytene stage, a distinct individuality (Figs. 58 and 59).

The presence of constant and characteristic differences among the

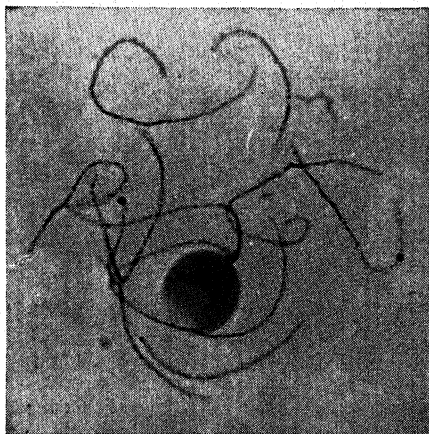


FIG. 57. The ten chromosome pairs with the nucleolus attached to the sixth chromosome at the pachytene stage of meiosis in the pollen mother cell of corn (*Zea mays*). (Courtesy of D. T. Morgan, Jr.)

chromosomes within a nucleus has led to the hypothesis that members of different pairs of chromosomes in a diploid complement carry qualitatively different materials, that is to say, different chromosomes contain different genes. A rigorous proof of this *hypothesis of chromosome individuality* was given only by studies on chromosomal aberrations carried out between 1920 and 1939. Nevertheless, this hypothesis played an important role in the development of genetics in the early years of the current century. The hypothesis should be understood in the sense that a chromosome can arise

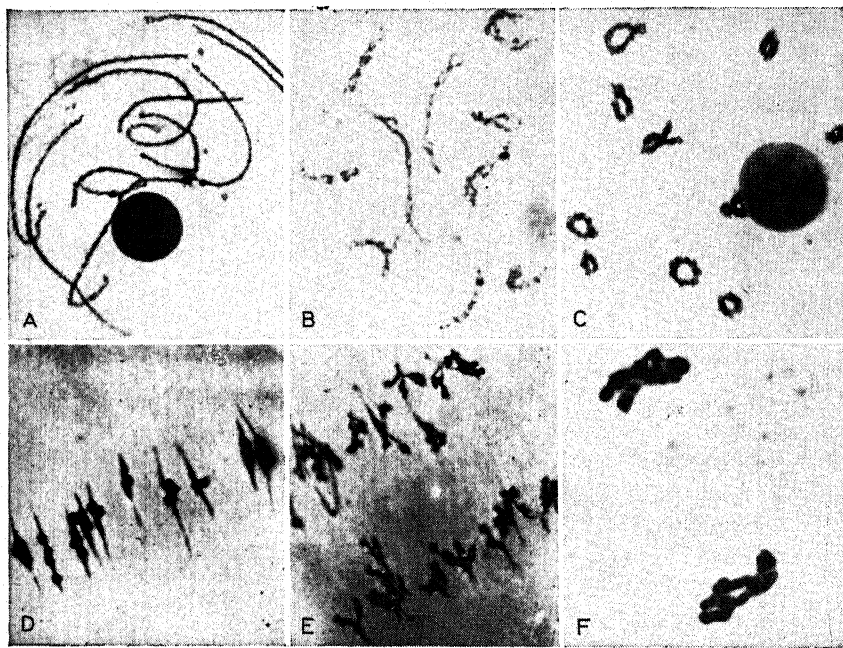


FIG. 58. Stages of meiosis in corn (*Zea mays*): A, pachytene; B, diplotene; C, diakinesis; D, metaphase; E, anaphase; and F, telophase of the first meiotic division. (Courtesy of M. M. Rhoades.)

only through a division of a previously existing chromosome containing the same genes. On the other hand, the appearance of a chromosome as seen in a microscope differs, of course, at different stages of cell division and also in cells of different tissues and parts of the body.

Meiosis. A very important basis for understanding the mechanism of heredity proved to be the studies on the behavior of chromosomes during the formation of gametes in animals and of spores in plants. These studies were initiated by Boveri (1887) and developed by Montgomery (1901), Janssens (1909), and others. Since a zygotic nucleus contains twice as

many chromosomes as do the gametic nuclei which unite at fertilization and since chromosome numbers remain constant from generation to generation, there must be a process which reduces the diploid chromosome complement back to the haploid number. This process, known as *meiosis*, occurs

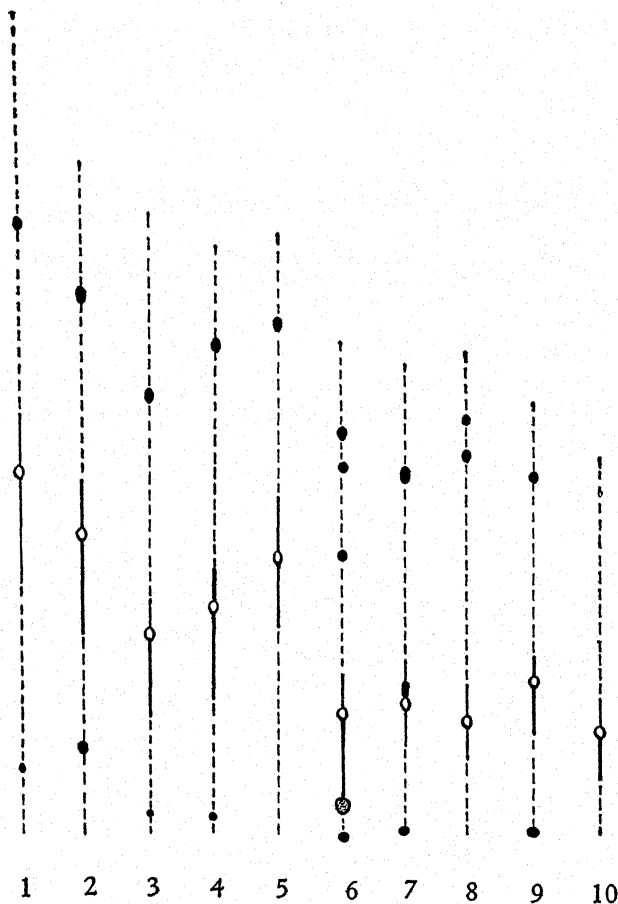


FIG. 59. Schematic representation of the chromosomes of maize illustrating relative lengths, positions of the centromeres (clear, oval-shaped), the deeply staining knobs (shaded), and the nucleolar organizer on the sixth chromosome (dotted). The heteropycnotic regions adjacent to the centromeres are represented by solid lines while the euchromatic portions of the chromosomes are shown by broken lines. (From M. M. Rhoades.)

in the sex glands, or *gonads*, during *spermatogenesis* and *oögenesis* (formation of male and female gametes) in animals and in the sporangia of higher plants incident to *sporogenesis* (spore formation).

Diploid cells, *spermatogonia* and *oögonia*, from which gametes are to arise

in animals, contain two of each kind of chromosome, being in this respect just like other body, or *somatic*, cells. The *spore mother cells* in plants are also diploid. The members of a pair of chromosomes which are alike in form and which carry similar genes are called *homologous chromosomes*, or

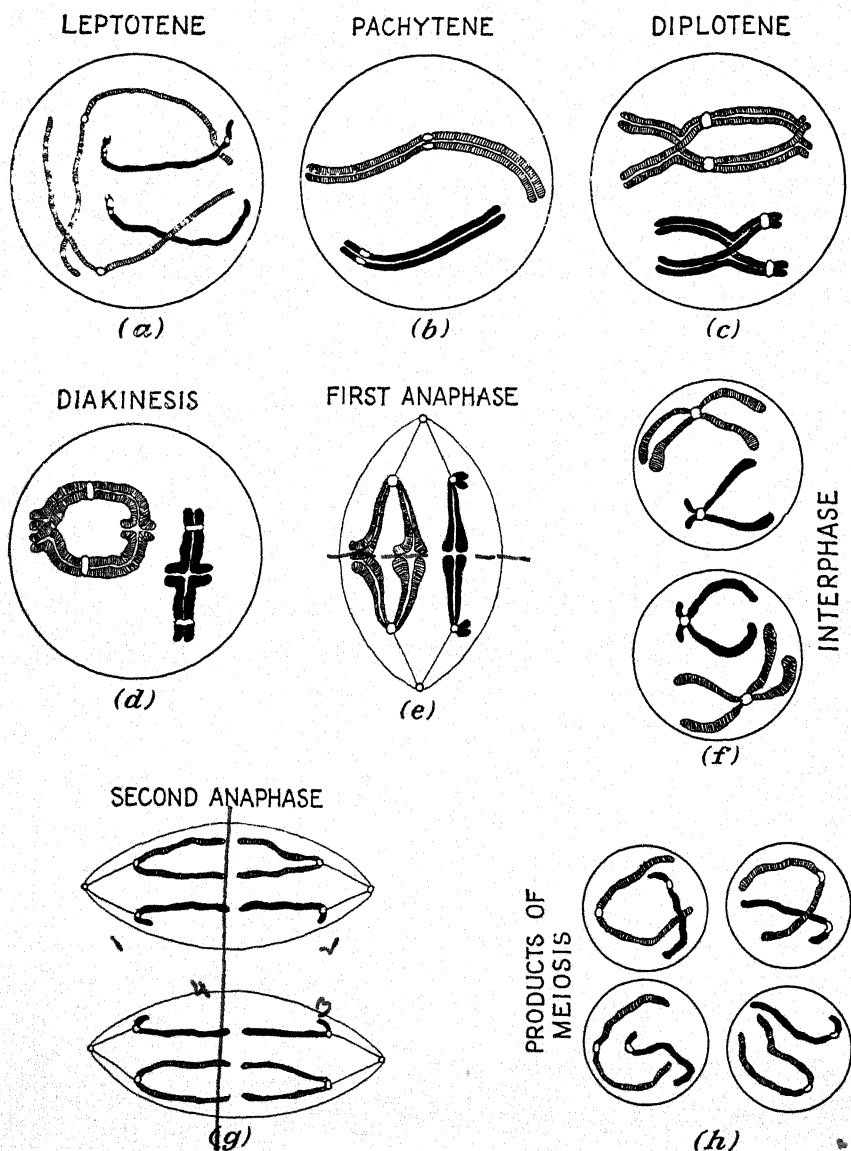


FIG. 60. Diagrammatic representation of meiosis.

homologues. The essence of meiosis is that at a certain stage the two members of each pair, that is, the homologues, seek each other out and undergo pairing, also known as *conjugation*, or *synapsis*, after which the conjugated chromosomes disjoin and go to different daughter cells. The result is the reduction of the number of chromosomes from the zygotic diploid $2n$ number to the haploid gametic n number. Synapsis and reduction occur in a very precise way which must be followed in detail from stage to stage.

As meiosis approaches, spermatogonia and oögonia increase in size, often manyfold, and become primary *spermatocytes* and *oöcytes*. The nuclei of these cells then enter a prophase during which the diploid number of chromosomes appears in each nucleus in the form of long, single, slender threads. This stage is known as *leptotene* (Fig. 60a). The homologous threads then come together in synapsis and unite in a haploid number of pairs, or bivalents (Fig. 60b), in which the threads contract and become shorter and thicker. This is the *pachytene* stage (Fig. 60b). Each chromosome in the bivalent then splits lengthwise into two halves, or chromatids. The four homologous chromatids thus formed remain united in a four-strand structure, or tetrad. This is the *diplotene* stage (Fig. 60c).

Something very important happens during the transition from pachytene to diplotene. The homologous chromatids break in one or more places, and the partner strands exchange parts so that new chromatids are formed consisting of sections of the two synapsed chromosomes. The chromosomes formed as a result of this consist of some parts which belonged to the maternal and some to the paternal homologous chromosomes (Fig. 60c). Under the microscope, one can see that the pairs of chromatids in the tetrad are held together at the places where two of the chromatids cross each other at the points where exchange of sections has taken place. Such cross-shaped configurations of chromatids are called *chiasmata* (singular *chiasma*). The number of chiasmata in each bivalent is variable. In some bivalents only one chiasma is formed, in others two or several, while in some exceptional cases no chiasmata have been observed.

Following diplotene, the tetrads become shorter and more compact (*diakinesis*, Fig. 60d), and in the metaphase of this first meiotic division the tetrads break up each into two dyads, which separate and pass to opposite poles of the division spindle (Fig. 60e). After a brief interphase, the second meiotic division occurs in which each dyad separates into two single chromatids (Fig. 60f to h), each of which passes to opposite poles.

The two meiotic divisions result, then, in the formation of four nuclei, each carrying one chromatid from each tetrad, that is, the haploid number of chromosomes. It must, however, be noted that, where chiasmata occur, the chromosomes resulting from meiosis may no longer be like the maternal and paternal chromosomes which underwent pairing at the pachytene



FIG. 61. Pachytene (or early diplotene) stage in a human spermatocyte. (Courtesy of J. Schultz.)

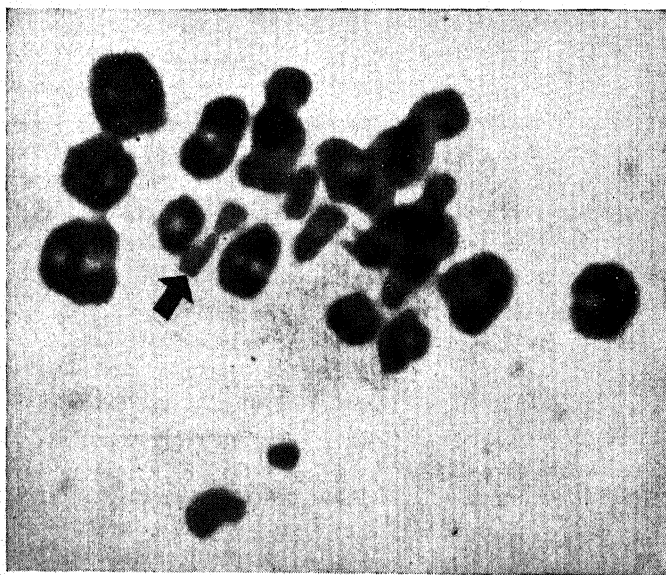


FIG. 62. Diakinesis in a human spermatocyte. The arrow indicates the X-Y chromosome bivalent. (Courtesy of J. Schultz.)

stage; instead, the chromosomes coming out of meiosis may be compounded of sections of the maternal and paternal homologues. Furthermore, the different (nonhomologous) maternal and paternal chromosomes undergo segregation independently of each other. As a consequence, a gamete formed by an individual will virtually never include all the maternal or all the paternal chromosomes. Far more commonly, it will contain various mixtures of maternal and paternal elements (Fig. 63).

In animals, meiosis results in the formation of four haploid nuclei which become the nuclei of gametes. In spermatogenesis in the male, each

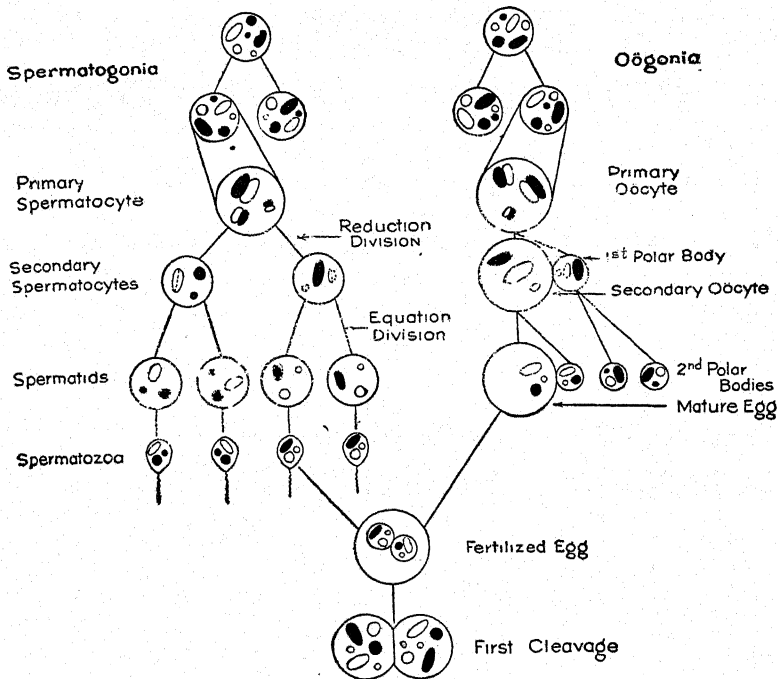


FIG. 63. Diagram of spermatogenesis and oögenesis in an animal. (After A. F. Shull.)

nucleus enters a *spermatid* cell, which then becomes transformed into a spermatozoon. In oögenesis in the female, three of the nuclei form polar bodies which usually degenerate, and the fourth becomes the female *pronucleus* of the egg cell. Fertilization restores the diploid condition, which then is retained in the somatic cells, as well as in the cells which give rise to the gonads.

Gamete Formation in Plants. The life cycle in most plants is more complicated than in animals, and the formation of gametes, instead of following meiosis directly, may be considerably deferred.

The lowest of the four main divisions in the plant kingdom, the thallophytes, show a considerable diversity in this regard. In most of the green algae, meiosis occurs in the first two divisions of the fertilized egg, and thus almost all the vegetative cells of the plant, as well as its gametes, have the haploid number of chromosomes. In the brown alga *Fucus* almost exactly the opposite is true, since meiosis takes place just *before* the formation of the gametes, and the cells of the plant body are thus all diploid, a condition essentially like that in animals. In many of the red algae the fertilized egg produces a group of spores (*carpospores*) each of which develops into a nonsexual plant, and these in turn bear nonsexual spores (*tetraspores*) in the formation of which meiosis is accomplished. The tetraspores develop into haploid sexual plants which ultimately bear gametes.

In plants above the thallophytes the "alternation of generations" is even more definite, a nonsexual diploid generation or plant, the *sporophyte*, bearing spores, in the formation of which meiosis occurs. These in turn grow into haploid sexual plants, *gametophytes*, which ultimately bear gametes, the fertilized egg developing into a sporophytic plant. There is thus an entire "generation" intercalated between meiosis and gamete production.

Among the seed plants, with which genetics has been chiefly concerned, the gametophytic generation has become very greatly reduced and is no longer an independent plant but is contained wholly within the reproductive structures of the sporophyte, which is the "plant" which we see. These reproductive structures are known as *flowers* (Fig. 64). Each consists, typically, of four sets of structures. Outside is a circle of protective parts, the *calyx*, and within this another circle of conspicuous and attractive parts, the *corolla*. Next occurs a series of "male" sexual organs, the *stamens*, each bearing an *anther*, which produces within itself a mass of single-celled *pollen grains*. Strictly speaking, however, the anther is not a sexual organ but is a sporangium, and the pollen grains are really microspores and not gametes, although they give rise directly to gametes. In the center of the flower is the "female" organ, the *pistil*, consisting of an *ovary*, *style*, and *stigma*. Within the ovary are one or more *ovules*, which, after fertilization, develop into seeds. The ovule is really a sporangium, also, and produces within itself a megaspore, which develops into a very much reduced female gametophyte, or "embryo sac," containing at least one egg cell, the true female gamete. The pollen grain (microspore), when it germinates on the stigma of the same flower or another of the same species, gives rise to a small group of cells (in the higher plants only three) which represent the last vestige of the male gametophyte. From the pollen grain develops a *pollen tube* which penetrates the style and enters

the ovule in the ovary. Down this tube pass the contents of the pollen grain—one nonsexual nucleus and two other nuclei, the true male gametes. One of these gametes unites with the egg cell in the ovule, and from this fertilized egg develops the *embryo* of the seed. The second male nucleus unites with the endosperm nucleus and gives rise to the endosperm tissue of the seed, which thus has three members of each chromosome set (triploid). This remarkable process of “double fertilization” (Figs. 64, 65) results in the formation of endosperm tissue, which partakes of both paternal and maternal inheritance; and in plants where the ovary wall and seed coat

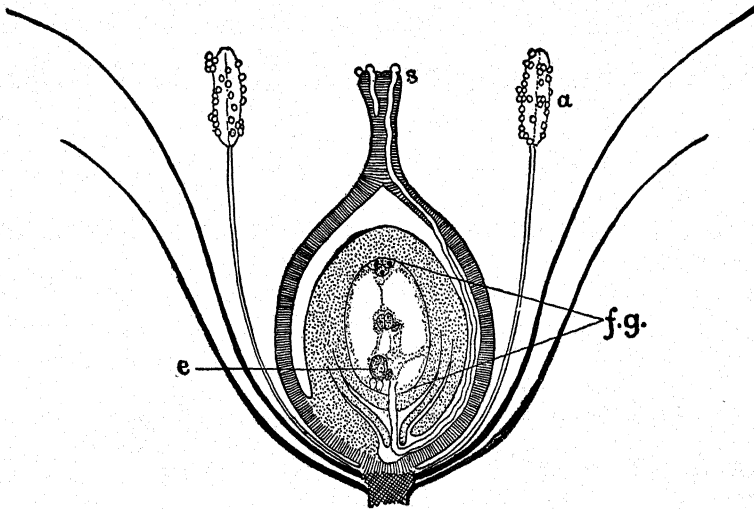


FIG. 64. Diagram of a vertical section through a flower, showing pollination and fertilization. The anthers, *a*, have opened, liberating pollen grains, two of which have germinated on the stigma, *s*. The pollen tube from one of these has grown down the style and carried the two male gametes to the embryo sac or female gametophyte, *f.g.*, of the ovule, where one is fertilizing the female gamete or egg, *e*. From the union of their nuclei will develop the embryo of the seed, which grows into a new plant.

are thin and transparent, as in the kernel of maize, a direct effect of the male gamete on the character of the endosperm is evident. Thus, if an ear of maize from a type normally bearing white endosperm is pollinated by pollen from a yellow race (yellow endosperm color being dominant over white), the endosperm of the seeds produced will be yellow. This direct effect of the male gamete on tissues other than embryonic ones is known as *xenia*.

The Parallelism between the Behavior of Chromosomes and Genes. In this chapter the essentials of the processes have been described by which the chromosomes carried in the diploid cells are distributed among the haploid gametes or spores. These processes can be followed by observa-

tion under a microscope. Now, it occurred to W. S. Sutton in 1902 (who was at that time a graduate student at Columbia University) and in the same year to the great German cytologist Boveri that the behavior of the

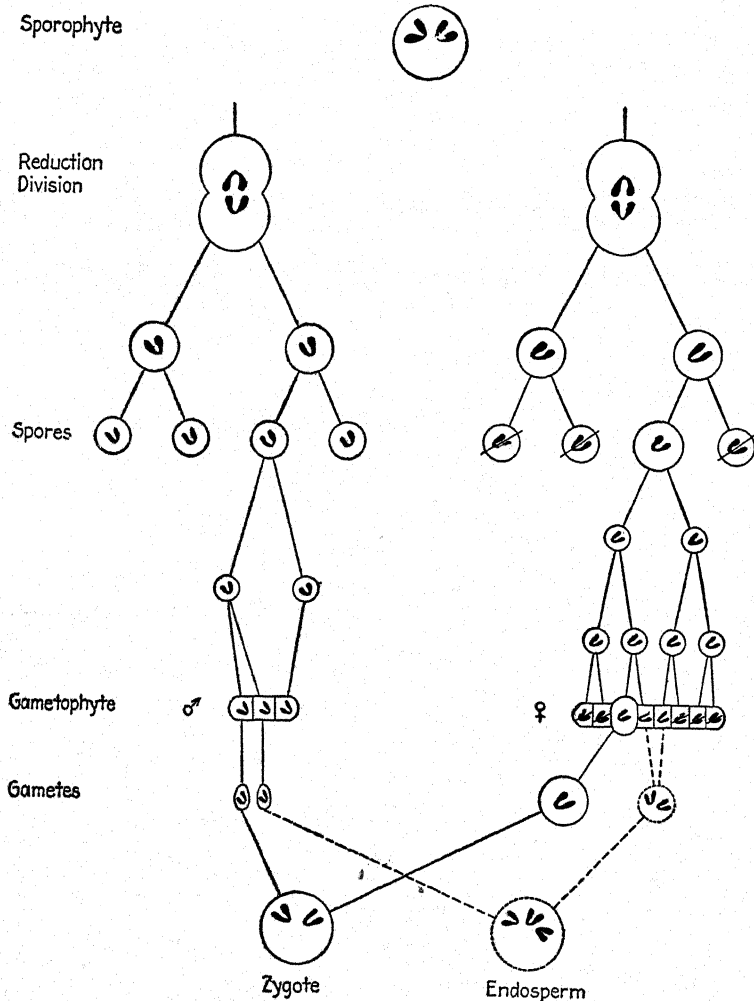


FIG. 65. The life cycle of an angiospermous seed plant. Diagram showing chromosome reduction in formation of microspores (left) and megaspores (right); gametophyte development; gamete formation; and double fertilization. (After Mohr).

chromosomes in the history of the formation of gametes and of fertilization resembles in a very striking way the behavior of the factors of inheritance, the genes, as inferred from the breeding evidence. The truth of the as-

assumptions concerning the behavior of the genes (Mendel's laws) is convincing, not because these units in the gametes have been seen but because the principles of segregation and independent assortment are the only hypotheses which satisfactorily explain the results of breeding experiments. The parallelism to be noted, then, is that which exists between a concrete set of facts (chromosome behavior) and the hypotheses proposed to explain another set of facts (gene behavior). Aside from the general similarity between these two processes, there are certain specific laws which apparently apply in a similar way to each.

1. Both the chromosomes and the genes behave in inheritance as though they were individual units. The individuality of the chromosomes is a matter of direct observation under the microscope. Each pair of chromosomes can be seen, in favorable material, to be different from every other pair. Each gene, likewise, has an individuality which is inferred from its indivisibility in inheritance and its emergence intact and unaltered after a cross.

2. The facts of inheritance can be explained only on the assumption that the genes which make up the genotype of every individual occur in *pairs* (allelic pairs) and that one member of each pair was contributed by one parent of this individual and the other by the other parent. This is precisely the situation observed in the case of the chromosomes, for these are also seen to be definitely associated in pairs, each member of which has been derived from one of the two parents.

3. Each gamete is seen to contain only one member of each pair of chromosomes, and each gamete likewise contains but one member of each pair of allelic genes. That the gametes contain the reduced or haploid number of chromosomes is known from actual chromosome counts, especially at the reduction division. That each gamete contains only one of a pair of genes was found to be a necessary inference from breeding experiments. In fact, the most important of Mendel's principles assumes a process of segregation by which, in the formation of gametes, each factor separates sharply from its alternative or allele, the two members of the pair always entering different gametes so that the gametes are "pure" genetically. Such a separation is actually found to take place between the two members of a pair of chromosomes at the reduction division, resulting in the inclusion of each member of the pair in different daughter cells (gametes). Both genes and chromosomes, then, undergo segregation, and in respect to both each gamete is pure, containing only one member of a pair.

Independent Assortment of Genes and Chromosomes. The pairing and disjunction of homologous chromosomes at meiosis furnishes a mechanism for the segregation of allelic genes (the first law of Mendel). The inde-

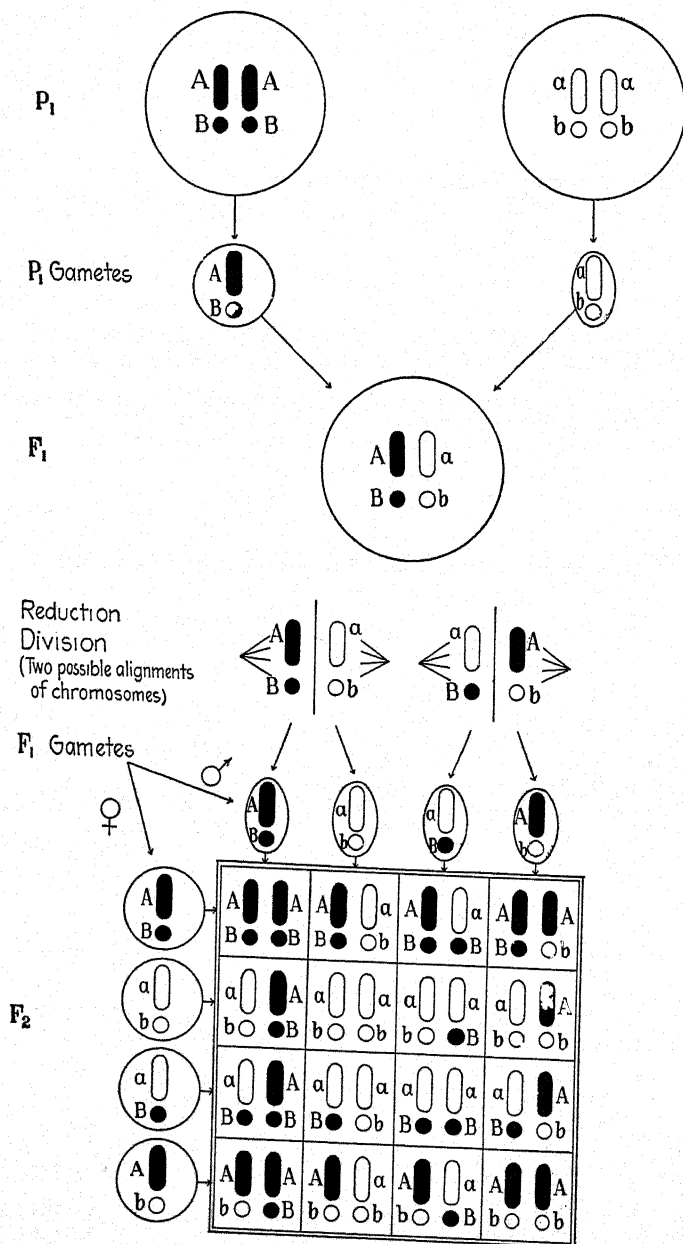


FIG. 66. Diagram showing independent assortment of two pairs of chromosomes, *A-a* and *B-b*. Note that at the reduction division there are *two* possible alignments of chromosomes producing *four* types of gametes. By random union these produce the sixteen different chromosome combinations shown in the F₂ checkerboard.

pendent assortment of genes (the second law of Mendel) can likewise be deduced from a knowledge of the chromosome behavior.

Diploid cells contain two sets of chromosomes, derived from the mother and father, respectively. Now, at meiosis the various pairs of chromosomes are assorted and distributed to the gametes independently of each other. A careful study of the diagram in Fig. 66 will show that it is a matter of chance whether two maternal or two paternal chromosomes go to the same pole at the reduction division or whether a maternal (black) chromosome happens to be associated with a paternal (white) one. The gametes may, therefore, contain any mixture of the maternal and paternal chromosomes. This is precisely the manner in which genes behave in inheritance, for in the formation of gametes by individuals heterozygous for two or more independent pairs of alleles it is purely a matter of chance

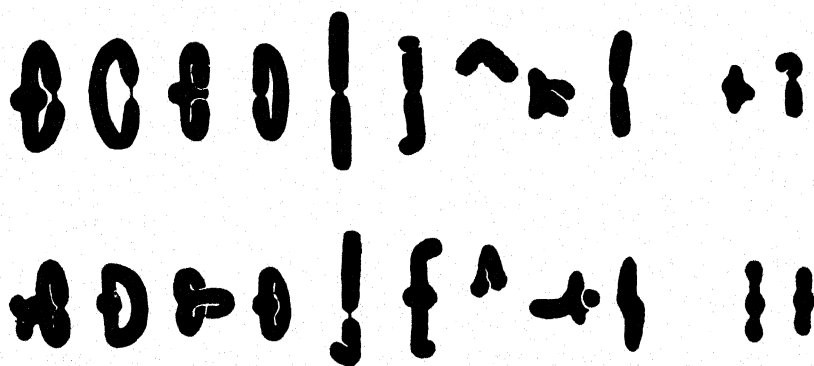


Fig. 67. Bivalents at the first meiotic division in the spermatocytes of two individuals of the grasshopper *Trimerotropis*. Some of the bivalents consist of unequal (heteromorphic) homologues. (After Carothers.)

as to how the members of the various pairs happen to become assorted and associated in the gametes.

Ordinarily it is not possible to distinguish the maternal and the paternal homologous chromosomes under the microscope. The chromosomes that pair at meiosis are usually identical in their cytologically visible features, even though they may contain different alleles of certain genes. In certain grasshoppers, Carothers found, however, some *heteromorphic homologues*, that is, chromosomes which pair regularly at meiosis but which differ in size and in shape sufficiently to be distinguishable. By observing the segregation of different pairs of heteromorphic chromosomes present in the same individual, Carothers found that different chromosomes assort independently (Fig. 67). This furnishes, then, the cytological evidence of independent assortment of maternal and paternal chromosomes.

In view of the close parallelism between the behavior of genes

and chromosomes, it is not surprising that Mendelian segregation and assortment are coextensive with the meiotic mechanism described in this chapter, that is to say, wherever segregation of genes is found, a mechanism of this sort may be inferred and has generally been found. Segregation occurs when the homologues separate and enter different daughter cells; the meiotic divisions may hence be known as the segregation divisions.

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PROBLEMS

194. What explanation can you suggest for the presence of polar bodies in the development of the animal egg?
195. If a character is found to be transmitted only by the mother, in what part of the gamete is it probably transmitted?
196. If the germ cells were formed by direct division of the nucleus without mitosis, how would this affect the character of the gametes and the process of inheritance?
197. What would happen to the chromosomal constitution of the nucleus if the reduction division did not take place?

198. What do you think would happen in the gametogenesis of a species with an odd number of chromosomes?

199. Of what advantage in plant reproduction is the complicated system of accessory structures such as the calyx and corolla?

200. In some groups of animals and plants the chromosome number of one species is a multiple of that in another. Thus in wheat some species have 14, some 28, and some 42 chromosomes. What does this suggest as to the evolutionary history of these species?

201. In the *Drosophilidae* (the family to which the vinegar fly belongs) Metz has found that some species have 8 chromosomes while other related species have 6, 8, 10, and 12 chromosomes each. Assuming that all species are descended from an 8-chromosome type, how would you account for the species with more and with fewer chromosomes?

202. At the time of synapsis preceding the reduction division, the homologous chromosomes align themselves in pairs, and one member of each pair passes to each of the daughter nuclei. Assume that, in an animal with four pairs of chromosomes, centromeres A, B, C, and D have come from the father and A', B', C', and D' have come from the mother. In what proportion of the germ cells of this animal will all of the paternal centromeres be present together? all of the maternal?

203. If a given character *A* pertains to the *gametophyte* and the gametophyte of one plant shows it while that of another plant shows its allele *a* and if gametes from these two gametophytes unite, what will be the appearance of the succeeding generation of gametophytes with respect to this character?

204. If one gametophyte displays characters *A* and *B* and is crossed with another which displays *a* and *b*, what will the next gametophyte generation look like with respect to these two characters?

205. In the honeybee, unfertilized eggs may develop by parthenogenesis, in which case they produce males (drones). The fertilized eggs produce females (workers or queens). In spermatogenesis in bees there is no reduction division. If the females contain 32 chromosomes in the body cells and if oögenesis is the same as in other species, how many chromosomes would you expect to find in the body cells of the males?

206. A queen bee heterozygous for a dominant character mates with a drone which shows the same character. What characters would you expect the male and female offspring to show?

207. In maize there are 10 pairs of chromosomes in normal sporophyte tissues. What number would you expect to find in (a) endosperm; (b) pollen-tube nucleus; (c) embryo sac; (d) leaf; (e) root tip; (f) embryo of seed?

208. If rows of sweet corn, homozygous for the recessive gene for sugary endosperm, *sn sn*, are planted alternately with starchy corn, *Sn Sn*, some seeds on the sweet corn plants are found to have starchy kernels. Frame a hypothesis to account for this, and indicate how you would test it.

209. In a species with 10 pairs of chromosomes, a plant is found in which one of the homologues of pair 9 has a knob while the other has none; the members of pair 5 are also heteromorphic, one of them having a terminal satellite. Predict the chromosomal constitutions of the gametes and the progeny of this plant produced by self-fertilization.

CHAPTER VIII

GENES AND CHROMOSOMES

The hypothesis of Sutton and Boveri that the behavior of chromosomes at meiosis provides the mechanism for segregation and independent assortment of genes, *if it is assumed that the genes are located in chromosomes*, has been amply verified by subsequent research. Microscopic observations on cell division, fertilization, and meiosis have shown that despite multiform variations in detail these processes are remarkably similar in essential features throughout animals and plants, while the phenomena of Mendelian segregation, from which the existence of genes is inferred, have also been observed in all groups of organisms which reproduce sexually. The chromosome theory of heredity, as it came to be called, was however proved not by the general parallelism to which the earlier workers had called attention, but by new data obtained from breeding experiments accompanied by cytological examination of the same material. The crucial evidence was that in which an association was proved between a specific gene and a specific chromosome identified under the microscope. In this chapter we shall examine some of the proofs for the chromosome theory, beginning with the evidence that certain genes in *Drosophila melanogaster*, the vinegar fly, are located in the same chromosome which is chiefly concerned with sex-determination

Sex Chromosomes. We must now take account of one important exception to the statements made in Chapter VII, that (1) the chromosomes in diploid cells appear in pairs of homologous mates and that (2) the disjunction of the members of these pairs at the reduction division in meiosis results in *all* gametes receiving exactly equivalent chromosome complements. The discovery of this exception led to the development of the chromosome theory of sex determination, which will be discussed on its own merits in Chapter XV. In the present context, we are interested in sex chromosomes only in so far as they throw light on the problem of the association of chromosomes and genes.

Most of the higher plants and some of the lower animals are *monoecious*, or hermaphroditic, that is, the same individual produces both female and male gametes; and in such organisms, all of the chromosomes normally occur in pairs. On the other hand, most animals and some plants are *dioecious*, or *bisexual*, and in them eggs and sperm are produced by different

individuals, females and males. In some of the bisexual organisms, females and males differ visibly in chromosomal constitution. This was shown in the early years of this century by several investigators, among whom Montgomery, McClung, Sutton, Stevens, and especially Wilson must be mentioned.

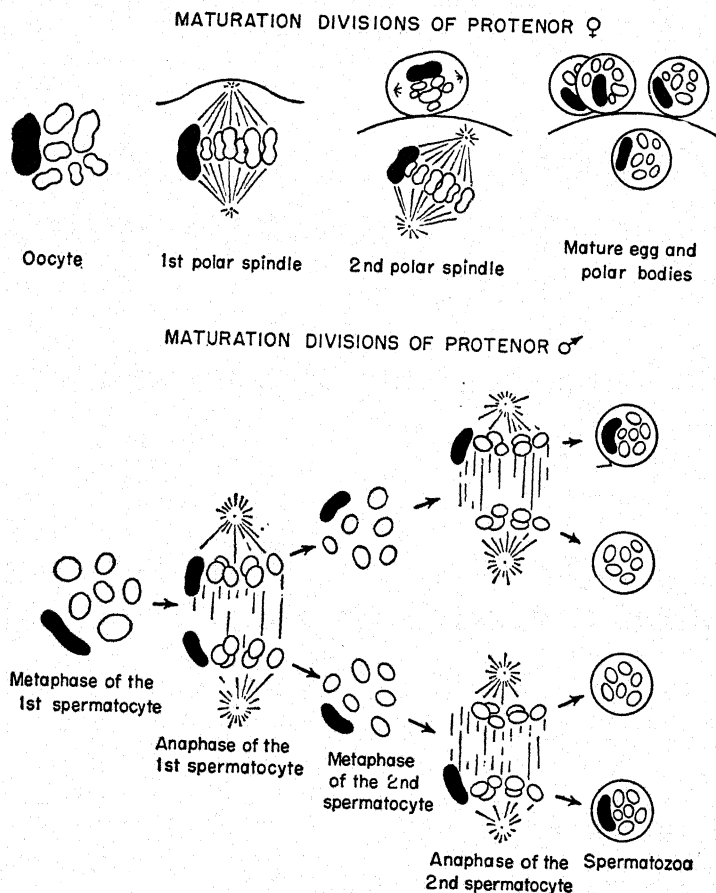


FIG. 68. Behavior of sex chromosomes at meiosis in the bug *Protenor*. The X chromosomes are shown in black. All eggs are alike in having a single X chromosome. Sperms are of two kinds—with and without an X chromosome. (From Morgan.)

The precise form which the chromosomal differences between the sexes take is not the same in different organisms. Perhaps the simplest situation is found in some grasshoppers, bugs, and other insects, in which the males have one chromosome less than the females (Fig. 68). For example, in the squash bug, *Anasa tristis*, Wilson found that females regularly have

22 chromosomes in their somatic cells, which unite into 11 pairs (bivalents) at meiosis. All eggs receive, therefore, a haploid set of 11 chromosomes. Males, however, have only 21 chromosomes in diploid cells, which form at meiosis 10 pairs and one odd unpaired chromosome. At the reduction division, the unpaired chromosome passes undivided into one of the two daughter cells, and therefore two kinds of spermatozoa are formed in equal numbers, some with 11 and others with 10 chromosomes. Fertilization of an egg (11 chromosomes) by a sperm with 11 chromosomes produces a *female* (22 chromosomes); fertilization by a sperm with 10 chromosomes produces a *male* with 21 chromosomes. The odd chromosome thus determines the sex of the individual which receives it, and it was consequently called the *sex chromosome*, or X chromosome. The other chromosomes which are alike in males and females have been called *autosomes*. The case may thus be formulated:

♀ = 10 pairs of autosomes + 2 X chromosomes (or ♀ = 10 AA + XX)

♂ = 10 pairs of autosomes + 1 X chromosome (or ♂ = 10 AA + X)

In many other animals and in several plants males and females have been found to differ, not by the presence or absence of one whole chromosome, but by the presence in one sex of a chromosome which is unlike its mate and unlike any chromosome in the opposite sex. In *Drosophila melanogaster* the female has four pairs of chromosomes (Fig. 69), the members of each pair being alike.

In the male there is only *one* of the straight rodlike chromosomes, the place of the other member of this pair being taken by a rod with a hook-shaped or bent end. The rodlike member, which is alike in both male and female, is the sex, or X, chromosome; the unlike member of this pair in the male is known as the Y

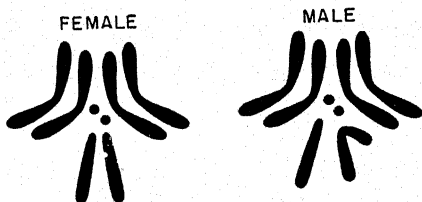


FIG. 69. The diploid chromosome complement in female and male *Drosophila melanogaster*.

chromosome. The eggs all have four chromosomes (3 A + X); the sperms also have four chromosomes, but half have the rod-shaped X chromosome (3 A + X), and half have the bent Y chromosome (3 A + Y). Fertilization of any egg by an X-containing sperm produces a female (6 A + XX); fertilization by a Y-containing sperm produces a male (6 A + XY).

The type of sex determination in which the female has two X chromosomes and the male has one X and one Y chromosome, is very widespread, being found not only in many insects and other invertebrates but also in some fish, in mammals including man, and in many dioecious plants. In

man, for example, somatic cells usually have 48 chromosomes, which in the female must form 24 pairs at meiosis ($23\text{ AA} + \text{XX}$). Each egg carries, then, 23 autosomes and an X. In the male, there are also 48 chromosomes; they form at meiosis 23 pairs in which the homologues are visibly alike and a pair consisting of members of unequal size, the larger one being the X and the smaller the Y chromosome ($23\text{ AA} + \text{XY}$). Two kinds of spermatozoa are formed, half of which carry an X ($23\text{ A} + \text{X}$) and the other half a Y chromosome ($23\text{ A} + \text{Y}$). The sex of the offspring is determined at fertilization by the kind of spermatozoon which happens to reach and enter the egg; the X-bearing sperms produce girls and the Y-bearing ones give rise to boys.

In all of the above cases the male is the *heterozygous*, or *heterogametic*, sex, because two kinds of sperm are produced, and the female is *homozygous*, or *homogametic*, because all eggs are sexually alike. But in some animals the relations are reversed, that is, the female has an unlike pair of chromosomes (XY), while the male is XX. For such cases a different formulation has often been used: the sex chromosomes have been called Z instead of X and the other member of this pair W instead of Y. Animals of this type are thus called ZW and ZZ, or, briefly, of the ZW type, to distinguish it from the *Drosophila*, or XY, type. We adopt here a similar terminology for both types, since the letters are merely symbols and do not indicate homology between X chromosomes in different forms. The two types may be distinguished as male heterogametic and female heterogametic, respectively.

In the domestic fowl, for example, the female lays eggs of two sorts, half with an X chromosome, which when fertilized by any sperm develop into males (XX), and half without an X chromosome, which develop into females. The mechanism is the same as in *Drosophila*, that is, two types of gametes are formed by one sex and only one by the other sex; and sex is determined at fertilization by the kinds of gametes which unite.

The sex-chromosome constitution of a number of animals was worked out in the period 1903 to 1910, and in most cases the male was found to be the heterogametic sex. As an exception two English investigators, Doncaster and Raynor (1906 and 1908), found a new type of inheritance in the currant moth *Abraxas* which could only be explained on the assumption that the female was heterogametic for a certain factor determining both a wing-pattern difference and sex. The same type of inheritance was found in the transmission of the black and white barred feather pattern of Plymouth Rock fowls by Spillman in 1909 (Figs. 75, 76, pp. 186, 187). Later the females of *Abraxas* and certain other species of moths were shown by cytological evidence to be heterogametic so that two main types of sex-chromosome constitution were established, male heterogamety in

many bugs, Diptera, some species of fish and amphibia, and mammals; female heterogamety in moths, birds, and certain fish.

Sex-linkage in *Drosophila*. Events of crucial importance for the chromosome theory proved to be the discovery of sex-linked genes in *Drosophila* by Morgan in 1910 and coordinated genetical and cytological study of these cases by Morgan, Bridges, and others. In the course of breeding experiments with the normal wild type, which has red eyes, Morgan found one individual in which the eyes were white. This gave rise to a true-breeding race of white-eyed flies. When he crossed this new variety with the wild, red-eyed type, the results from a cross of a white male by a red female were quite different from those obtained from the reciprocal cross of red male by white female. The results were found to depend on the sex of the parent in which the trait was introduced into the cross, whereas with other characters, as has been seen, it makes no difference in either the F_1 or the F_2 whether a given character is brought in by the male or female parent. The details of these experiments, which have been repeated many times, are shown in Figs. 70 and 71. From the cross of white-eyed male with red female the first-generation flies are red-eyed in both sexes (Fig. 70). When these are bred together, white reappears in a quarter of the F_2 offspring, indicating that red and white eye color are due to an allelic pair of genes of which red acts as the dominant. However, of the F_2 offspring all the females are red, while half of the males are red and half are white. The white male has transmitted his eye color only to his grandsons. These F_2 white-eyed males evidently carry no factors for red, since when bred with pure white stock no red-eyed individuals ever appear among their offspring. The females, however, are apparently of two kinds, genotypically. When bred with pure red males, half of them give nothing but red offspring and are thus pure for red, but the other half must carry the recessive white, for in their offspring half the males are white-eyed. When a red male is bred to a white female, however, quite a different result follows (Fig. 71). Among their F_1 offspring all the females are red-eyed, and all the males are white-eyed. When these are bred together, their offspring (the F_2) consist of red-eyed and white-eyed individuals in about equal numbers in both sexes. All the white-eyed flies are apparently pure, for no red-eyed flies appear in their offspring; and the red-eyed males bred to pure red females also produce only red-eyed descendants. The red-eyed F_1 females, however, must be heterozygous, for when bred to either white or red males, half of their male offspring are always white-eyed.

A typical sex-linked trait in *Drosophila*, such as white eye color, is found to follow a peculiar type of crisscross inheritance. A male transmits his sex-linked traits to his grandsons through his daughters. He

never transmits them to or through his sons. The trait thus seems to alternate or cross from one sex to the other in its passage from generation to generation. This of course is the mode of transmission followed by

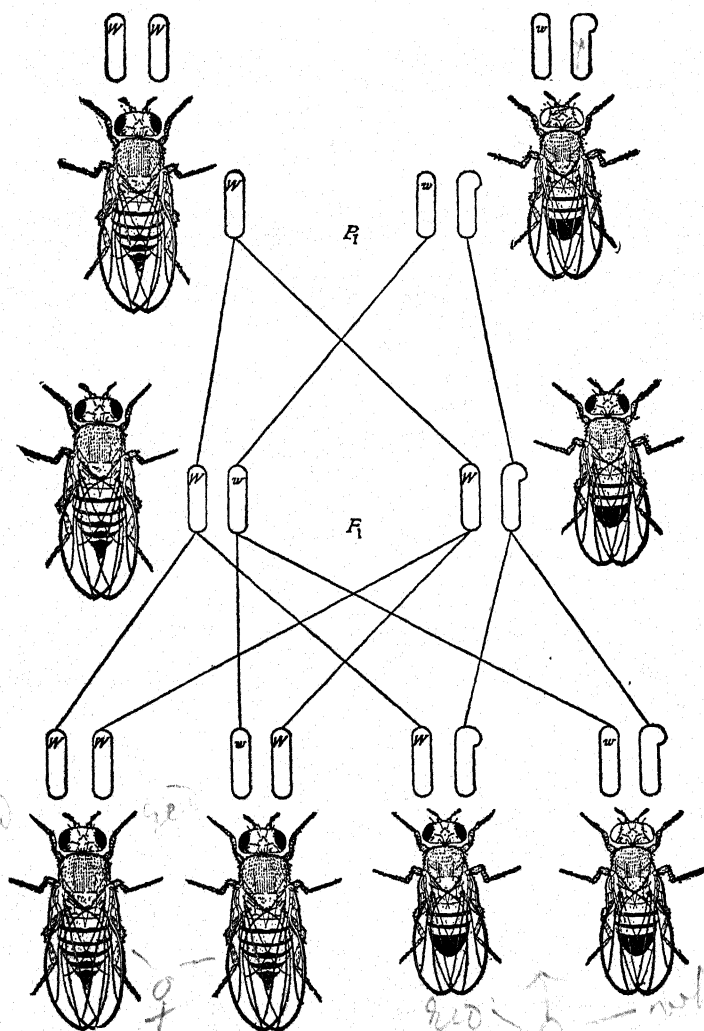


FIG. 70. Sex-linked inheritance in *Drosophila*. The cross of red-eyed female by white-eyed male. The course of the sex chromosomes carrying the sex-linked gene $W-w$ is traced from parents to F_2 . Females at left, males at right. (From Morgan, Sturtevant, Muller, and Bridges, courtesy of Henry Holt & Company.)

the X chromosome, as can be seen in the diagrams. Only the daughters get an X chromosome from the father, whereas both sons and daughters receive an X chromosome from the mother.

In explaining the peculiar inheritance of white eye color in *Drosophila*, it was assumed that the gene for white eyes is located in the sex chromosome and that the Y chromosome carries no normal allele for white. On

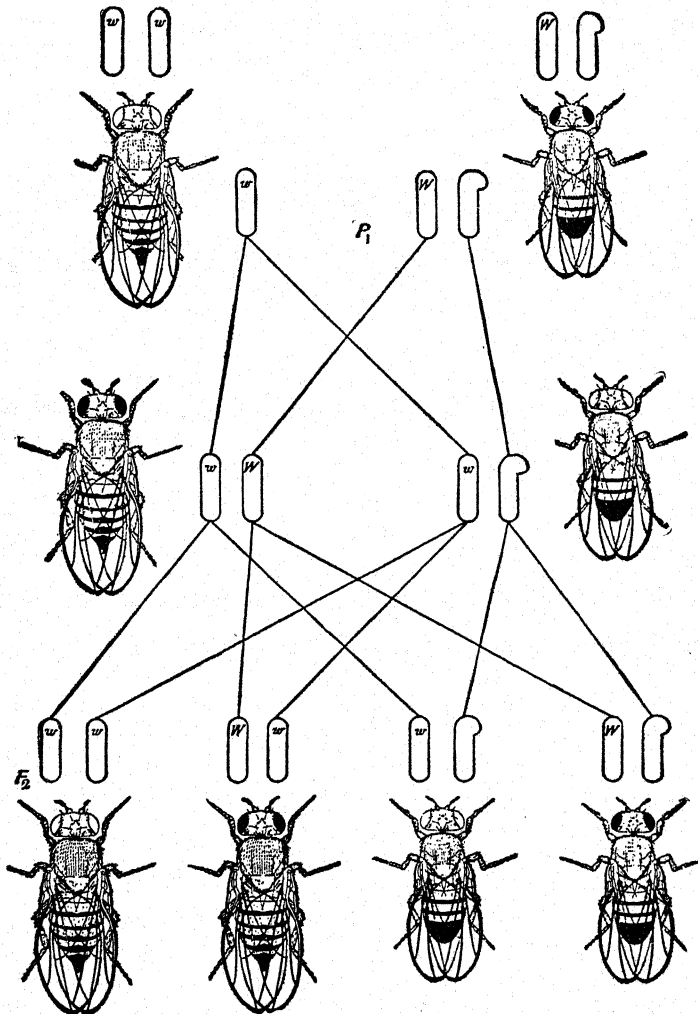


FIG. 71. Sex-linked inheritance in *Drosophila*. The cross of white-eyed female by red-eyed male, the reciprocal of the cross shown in Fig. 70. Females at left, males at right. (From Morgan, Sturtevant, Muller, and Bridges, courtesy of Henry Holt & Company.)

this assumption the case became clear, for the white-eyed female crossed with a red male transmitted a gene for white to each offspring and an X chromosome to each offspring. The daughters received also an X chro-

mosome from the father carrying the dominant allele of white and hence were red-eyed. The sons, however, received a Y chromosome from the father and hence no allele of white, and they were thus white-eyed. From the diagrams it is apparent that in all cases the gene for white follows exactly the transmission of the X chromosome. At least 140 other genes in *Drosophila melanogaster* follow the same mode of inheritance as white, which indicates that these genes are carried in the X chromosome.

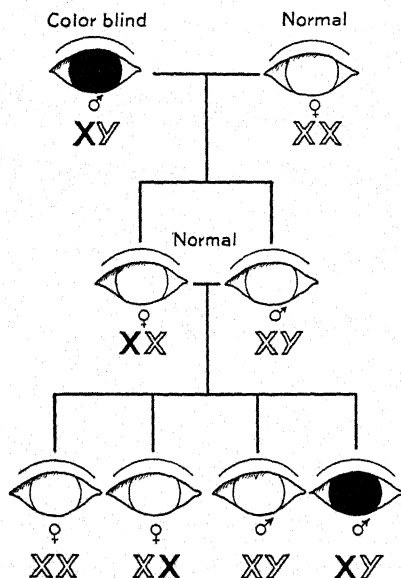


FIG. 72a The inheritance of color-blindness. A color-blind man mated with a normal woman. The defect is transmitted only through the daughters and appears in half of their sons, being carried in one of the X chromosomes. Color-blind individuals and chromosomes carrying the gene for this character are black. (From Dunn, courtesy of the University Society.)

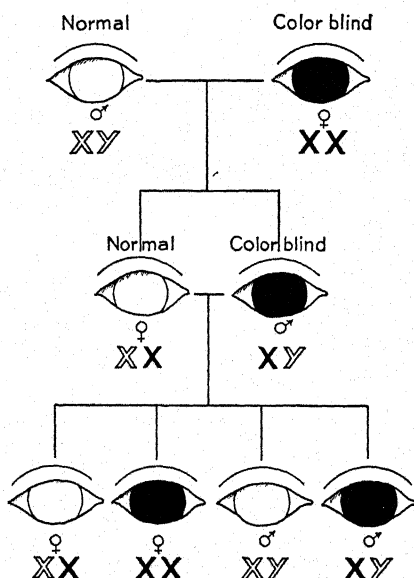


FIG. 72b. The inheritance of color-blindness. A color-blind woman mated with a normal man. The defect is transmitted to all the sons and (in the F₂ mating shown) to both grandsons and granddaughters. (From Dunn courtesy of the University Society.)

Sex-linked Genes in Man. Approximately 20 known genes in man are inherited like the gene white or its normal allele in *Drosophila* and therefore are presumed to be borne in the human X chromosome (Fig. 72).

The commonest sex-linked human trait is red-green color-blindness, which in the United States occurs in about 8 per cent of men and only about 0.5 per cent of women. Its peculiar mode of transmission chiefly to sons from the mother's family had been known for at least a hundred years, when E. B. Wilson in 1911 pointed out that all of the facts about the heredity of color-blindness could be explained by the assumption that

the recessive gene responsible for this condition is contained in the X chromosome and that in man the male is the heterogametic sex. These are just the assumptions made for the white-eye gene in *Drosophila* (Figs. 70 and 71).

It is easy now to see why color-blindness is found more often in men if one remembers that a father transmits his X chromosome to all of his daughters but to none of his sons, while a mother passes one of her two X's to all of her children. Therefore, all the sons of a color-blind mother are color-blind regardless of what kind of color vision her husband may have; but if the husband has normal vision, all of his daughters have also normal vision. These daughters are, however, carriers of the gene for color-blindness since they contain this recessive gene covered up by its dominant allele; married to men with normal color vision, they produce all normal girl children, but among the boys about half are normal and the other half color-blind. A color-blind daughter can be produced only if a color-blind man happens to marry a carrier, or a homozygous color-blind woman. Since color-blind men and women who are carriers or color-blind are less common than those with genes for normal vision, such marriages are rather rare.

Similarly, hemophilia is a disease restricted to men only, and such men are invariably sons of mothers who are themselves normal although carriers of the recessive hemophilia gene. The hemophilics, if they happen to survive and reach the reproductive age, produce daughters all of whom are themselves normal but are carriers of hemophilia, which they transmit to half of their sons (grandsons of the male hemophilics). One-half of the daughters of the female carriers are, of course, also heterozygous carriers. A female hemophilic could, theoretically, be produced if a woman who is a carrier marries a man who is a hemophilic; only two such marriages have been recorded in the literature, but the daughters whom they produced were normal. This led to a suspicion that the hemophilia gene may be lethal when homozygous. Yet this gene can be transmitted from a heterozygous carrier, to her daughters, granddaughters, etc., who have all normal blood and yet produce a half of their sons afflicted with hemophilia. A famous case of this sort is the transmission of hemophilia in some royal houses of Europe, which is traceable to Queen Victoria of England and her progeny (Fig. 73).

Heredity through the Y Chromosome. The sex-linked genes contained in the X chromosome of *Drosophila* have no alleles in the Y chromosome; this is the reason why a male which carries a single dose of a recessive sex-linked gene shows its effects in the phenotype. The Y chromosome is, therefore, genetically "empty" or "inert," at least compared with the X chromosome. One gene, bobbed, however, has alleles both in the X and

in the Y chromosomes. The recessive mutant allele of bobbed, when present in both X chromosomes of a female, causes the bristles on the body of the fly to be shorter and slenderer than normal. A male which carries bobbed in the X chromosome but a normal allele of that gene in the Y has normal bristles. If such a male is crossed to bobbed females, all the daughters are bobbed and all sons have normal bristles; the normal bristles are transmitted from father to sons, just as the Y chromosome is. Several bobbed alleles in the Y chromosome are also known, and males which carry bobbed both in the X and in the Y chromosomes show bobbed bristles.

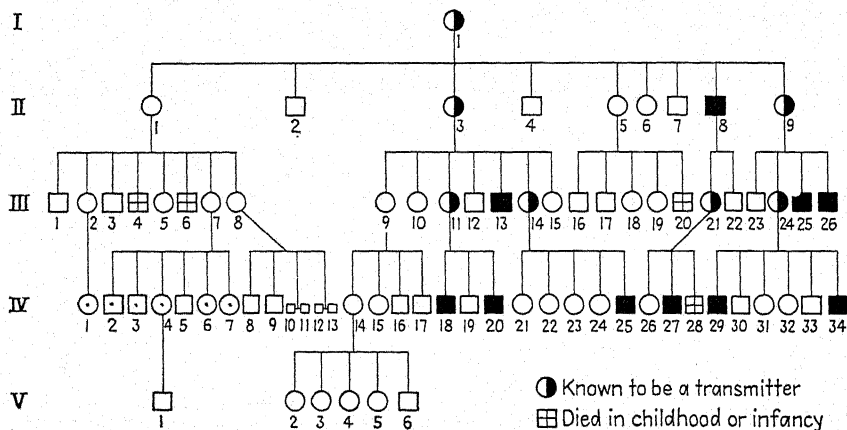


FIG. 73. Pedigree of hemophilia in the descendants of Queen Victoria of England. I-1, Queen Victoria; II-2, King Edward VII; II-3, Princess Alice; II-8, Leopold, Duke of Albany; III-11, Irene; III-13, Prince Frederick William of Hesse; III-14, Alexandra; III-21, Alice; III-24, Victoria Eugenie; III-25, Prince Leopold of Battenberg; III-26, Prince Maurice of Battenberg; IV-18, Prince Waldemar of Prussia; IV-20, Prince Henry of Prussia; IV-25, Tsarevitch Alexis of Russia; IV-27, Viscount Trematon; IV-29, Alfonso formerly Prince of Asturias; IV-34, Infante Gonzalo. (After Haldane.)

A curious abnormality in man, namely, a dense growth of long hair on the ears, and at least three other abnormalities have been observed in some pedigrees to be transmitted from fathers to all of their sons but to none of their daughters. These four genes are carried presumably in the Y chromosome. The work of Haldane and others has revealed the existence in man of at least five of the so-called partially sex-linked genes, which, like bobbed in *Drosophila*, seem to have alleles both in the X and in the Y chromosomes. The human Y chromosome does not seem to be quite so "empty" as that of *Drosophila*. These facts will be discussed further in the chapter on sex determination.

Sex-linked Genes in Poultry and in Moths. The kind of sex-linked inheritance described in *Drosophila* and in man is characteristic of animals

and of plants in which the male sex is the heterogametic one; this inheritance is readily understandable if the genes concerned are borne in the X chromosomes. A different kind of sex-linkage occurs where females are heterogametic, as is the case in birds, moths, butterflies, and certain other animals. In these cases, too, genes have been shown to follow the known course of transmission of the X chromosome.

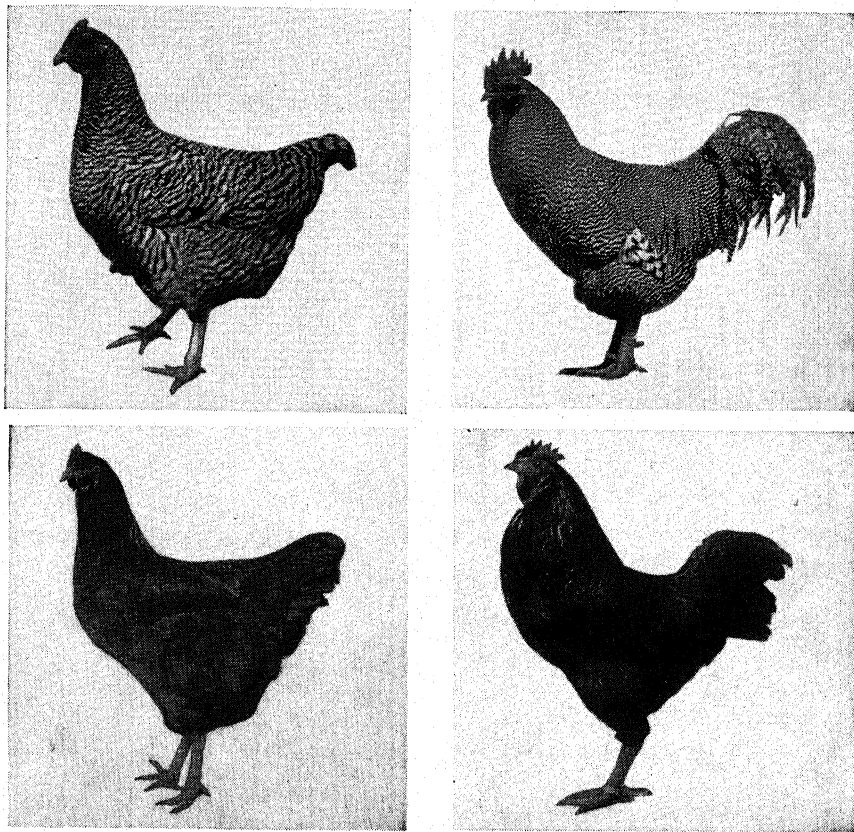


FIG. 74. Barring (above) and plain color (below) in females (left) and males (right) in domestic poultry.

The inheritance of the barred plumage in poultry is one of the best-known examples of this type (Fig. 74). The barred pattern, as seen in such breeds as the Barred Plymouth Rock, is dominant over black or red unbarred plumage. Breeding evidence indicates that a male may carry two genes for barring but a female only one; and cytological research has shown that there are two X chromosomes in the cells of the male but only one in those of the female. In the diagrams (Figs. 75 and 76) barred

plumage is represented by B and nonbarred by b . The cross between a nonbarred hen and a barred cock produces only barred offspring of both sexes. These when inbred produce only barred males in F_2 , but approximately half of the F_2 hens are barred, and the other half are nonbarred.

The reciprocal cross of barred hen and nonbarred cock gives, as might be expected, a very different result. Here the F_1 males are *all barred*,

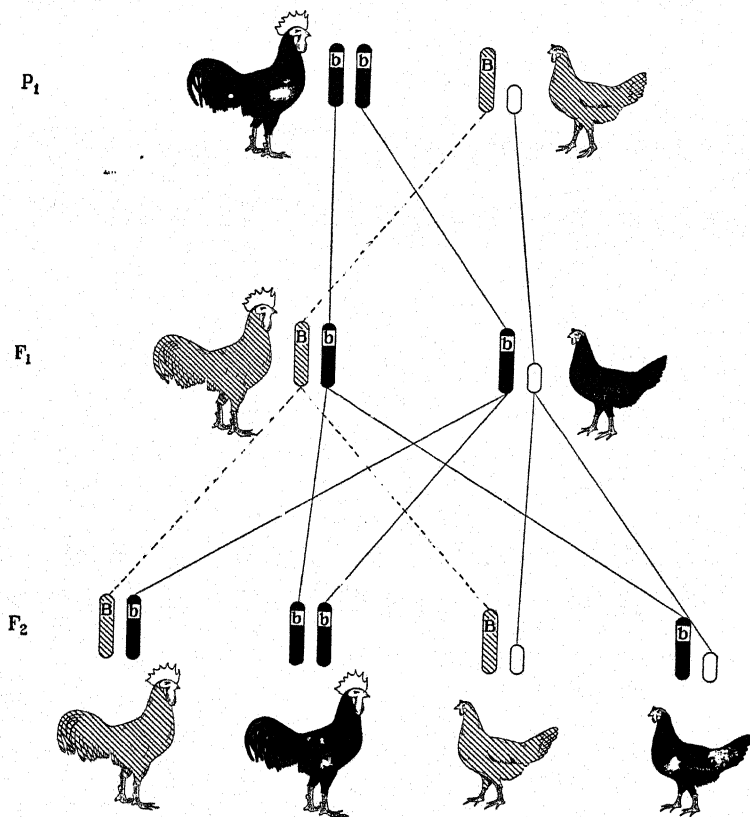


FIG. 75. Sex-linked inheritance in poultry. The cross of barred ♀ by nonbarred ♂ (See Fig. 76).

and the F_1 hens are *nonbarred*, while in F_2 there are equal numbers of barred and nonbarred birds in both sexes. Barring thus follows the same crisscross mode of inheritance as white eyes, except that in the fowl the sex-linked gene goes from mother (XY) to her sons only, while the father (XX) transmits it to both his sons and daughters. The gene follows the X chromosome in both cases.

In the fowl, pigeon, duck, canary, several species of moths, and one

species of fish, sex-linked characters have been studied and found to resemble barring in their inheritance.

An example of sex-linked inheritance in dioecious plants is described on page 397. In the few cases thus far discovered the male is the heterogametic sex.

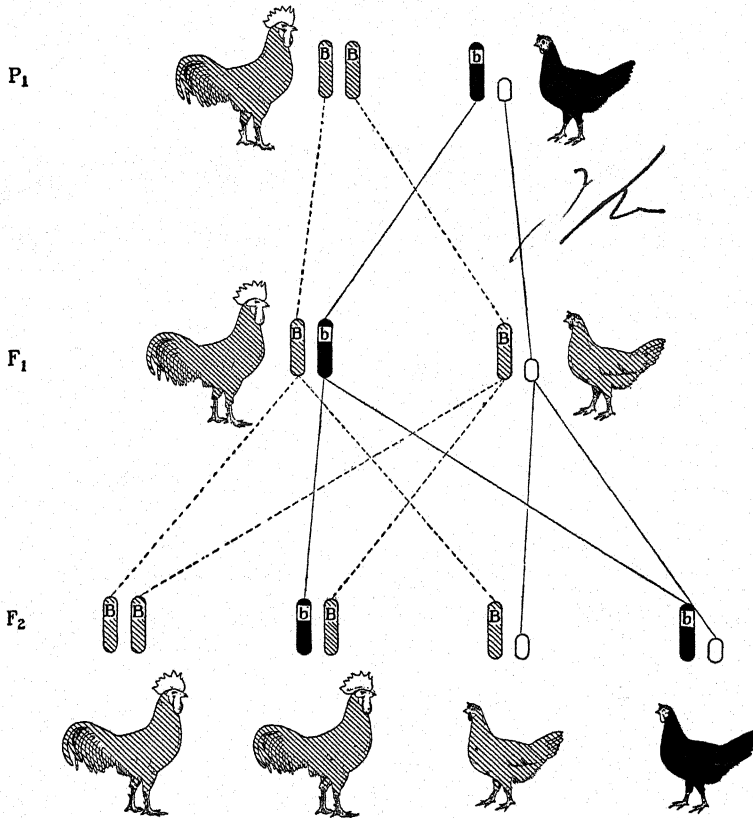


FIG. 76. Sex-linked inheritance in poultry. The cross of barred ♂ by nonbarred ♀. The course of the X chromosomes carrying the sex-linked gene *B* (barring) and its recessive allele *b* is traced from parents to F₂. Chromosomes carrying *B* are crosshatched; those carrying *b*, solid black. Males at left; females at right.

Attached X Chromosomes in *Drosophila*. A striking proof of the chromosomal theory of heredity was given by Bridges (1916). He found that certain exceptional females had got a sex-linked trait from their mother, and this seemed at first glance to contradict the view that the behavior of sex-linked genes parallels that of the X chromosomes in going from mother to son. He figured out what deviations from the normal distribution of X chromosomes could produce the exceptional results found in his crosses

and then proved by means of ingenious experiments and cytological observations that the predicted deviations were actually present. L. V. Morgan (1922) found another exceptional case, which later proved the same principle. The proof in this case is even more diagrammatic than in Bridge's original experiments, and we shall thus consider it first.

The yellow body color in *Drosophila melanogaster* is, like the white eye color described above, due to a sex-linked recessive gene. Yellow females crossed to normal brownish-gray males ordinarily give in F_1 all normal daughters and yellow sons and equal numbers of yellow and gray in both sexes in F_2 . Normal females crossed to yellow males give all normal offspring in F_1 , and in F_2 all normal females and a segregation 1 yellow : 1 normal among the males (*cf.* Figs. 70 and 71, p. 180). L. V. Morgan found, however, an exceptional yellow female which, when crossed to normal males, produced only yellow daughters and normal sons. These yellow daughters repeated the exceptional performance of their mother; the sons of the original exceptional mother were all sterile, but in subsequent generations the normal sons behaved like the usual normal flies.

What is the explanation of this exceptional behavior? As we know, a female has normally two X chromosomes, which disjoin from each other at meiosis, so that each egg receives a single maternal X chromosome. Now, let us suppose that in the exceptional yellow female the X chromosomes became permanently attached to each other, so that meiotic disjunction is no longer possible and *both* X's either remain in the egg or else are extruded in the polar body (Fig. 77). The original exceptional yellow female must, therefore, have produced two kinds of eggs: those with the attached X chromosomes carrying the gene yellow, and those without an X chromosome. Fertilized by the sperm of a normal male, the two kinds of eggs will give four kinds of zygotes, namely, (1) with two attached yellow X chromosomes and a normal X chromosome from the male, (2) with two attached yellow X chromosomes and a Y chromosome, (3) with a normal paternal X chromosome and a Y chromosome, (4) with two Y chromosomes and no X. The class (2) are the observed yellow daughters, and the class (3) are the observed normal sons. Zygotes (1) and (4) usually die, although zygote (1) sometimes survives and gives a so-called superfemale (see p. 381). The exceptional yellow females will henceforth carry a Y chromosome, and hence the two kinds of eggs produced will have either two attached X chromosomes or a Y chromosome, as shown in Fig. 77.

How can the validity of L. V. Morgan's hypothesis be tested? This hypothesis permits several predictions to be made, namely, (1) the females from the exceptional yellow stock must have their two X chromosomes somehow attached to each other, (2) they must carry not only two X's but

also a Y chromosome, (3) about half of their eggs are expected to produce no viable flies because some of them will have three X chromosomes (*duplication*) while others will have none and thus will be *deficient* for all X-chromosome genes. These predictions were tested and found to be valid, and the hypothesis was clearly verified. Females with attached X chromosomes have been found also in a related species, *Drosophila simulans*, by Kossikov (1934).

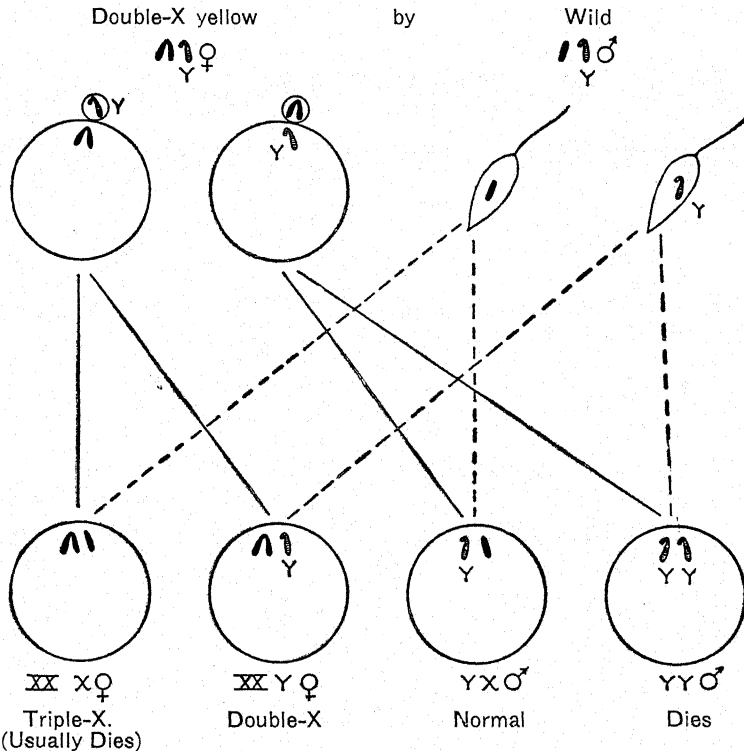


FIG. 77. Inheritance of "attached X" chromosomes in *Drosophila*. The X chromosomes of the mother each carry the gene yellow and these are transmitted together to all viable daughters. The only surviving sons are those which receive the Y chromosome from the mother and the X from the father. (After Morgan.)

Nondisjunction of X Chromosomes in *Drosophila*. White-eyed *Drosophila melanogaster* females crossed to red-eyed males normally produce red-eyed daughters and white-eyed sons. Bridges found that exceptional white-eyed daughters and red-eyed sons nevertheless occur in such crosses, one exceptional fly per 2,000 to 3,000 regular offspring. In trying to explain such exceptions, Bridges supposed that the two X chromosomes present in a female even without being attached to each other may occasionally fail to disjoin at the meiotic division. This *primary nondisjunction*

tion leads, then, to production of eggs with two X chromosomes and with no X chromosome (Fig. 78). Fertilized by normal sperm, four types of zygotes will be produced, namely, (1) with three X chromosomes, (2) with two X's and a Y chromosome, (3) with an X but no Y, and (4) with a Y but no X. Zygotes (1) usually die, and (4) always die. If the mother had white eyes, zygote (2) will be a white-eyed female and zygote (3) a red-eyed male. The exceptional red-eyed males are sterile and have no Y chromosome.

When the exceptional white-eyed females with a Y chromosome (these may be called XXY females) are again crossed to unrelated normal males with red eyes, about 96 per cent of the daughters have red and about 4

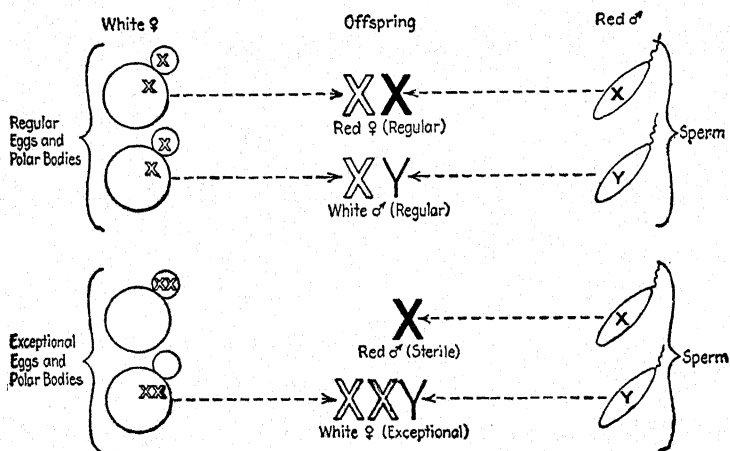


FIG. 78. Nondisjunction of the X chromosome in *Drosophila melanogaster*. Cross of white ♀ by red ♂. X chromosomes carrying white in outline; those carrying normal allele (red) shown in solid black. (After Morgan.)

per cent have white eyes, while among the sons about 96 per cent are white-eyed and 4 per cent are red-eyed. The production of the white daughters and red sons is due to *secondary nondisjunction*, as shown schematically in Fig. 79. At meiosis in XXY females, the X chromosomes disjoin in about 92 per cent but pass together either in the polar body or in the egg nucleus in about 8 per cent of the oöcytes. Four kinds of eggs are formed, namely, (1) with a single X chromosome, (2) with an X and a Y chromosome, (3) with two X chromosomes, and (4) with a Y chromosome. Fertilized by the sperm of a normal red-eyed male, these eggs should produce the eight types of zygotes shown in Fig. 79. Examination of this figure will disclose ways for testing the validity of this seemingly very complicated explanation. In the first place, not only all white-eyed females but also some of the red-eyed ones must carry not only two X's

but also a Y chromosome; some of the white-eyed males must belong to a hitherto unknown type carrying an X and two Y chromosomes; and the exceptional red-eyed males must, in contrast to those arising from primary

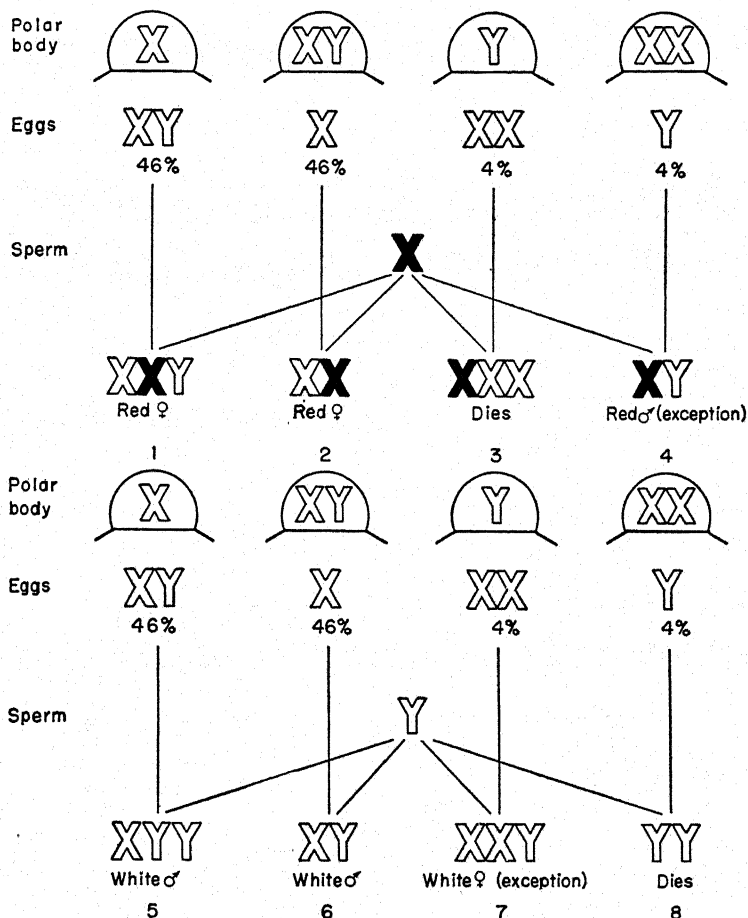


FIG. 79. Secondary nondisjunction in *Drosophila melanogaster*. A white-eyed female having an extra Y chromosome (XXY female) is crossed to a normal red-eyed male. In the upper part of the diagram the fertilization of the four possible kinds of eggs by spermatozoa containing an X chromosome is shown. In the lower part of the diagram the fertilization of the same eggs by spermatozoa with Y chromosomes is shown. (From Morgan.)

nondisjunction (see above), have a Y chromosome and accordingly ought to be fertile. These predictions were indeed all verified by Bridges.

The phenomena of nondisjunction have been observed for other sex-linked genes in *Drosophila melanogaster*. Bridges's masterly analysis of

these phenomena was of crucial importance in the history of genetics, since it proved beyond reasonable doubt that the sex-linked genes are actually carried in the X chromosome, a fact which had been inferred but not proved from observations on normal sex-linked inheritance before Bridges.

Trisomics and Monosomics in *Drosophila*. We have seen that nondisjunction of X chromosomes in *Drosophila* leads to the appearance of zygotes which have one chromosome more (XXX and XXY females and XYY males) or one chromosome less (X) than normal flies. Since the Y chromosome contains relatively few genes, flies with extra Y's appear to be normal, while males which lack a Y differ from normal only in being sterile. On the contrary, the presence of an extra X chromosome (XXX zygotes) is usually lethal.

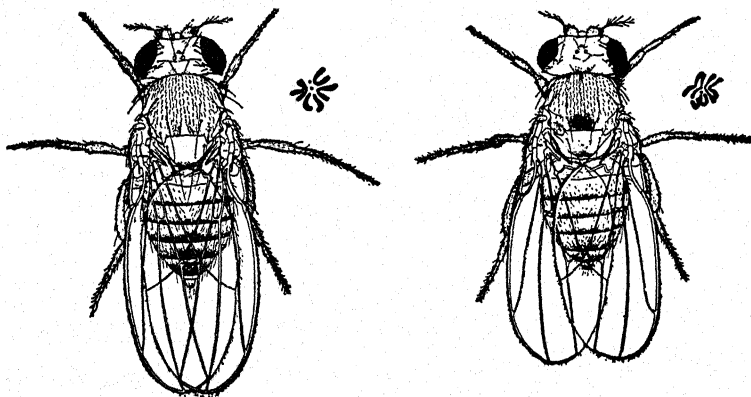


FIG. 80. Normal (left) and haplo-IV individuals of *Drosophila melanogaster*. Their respective chromosome groups are shown at the right of each. (From Bridges.)

Nondisjunction takes place occasionally not only for the X and Y but for other chromosomes as well. It results in the production of zygotes which have one of the chromosomes of the normal complement in triplicate (*trisomics*, or $2n + 1$ types) or which have a chromosome represented only once instead of twice (*monosomics*, or $2n - 1$ types). Individuals with one or more reduplicated or missing chromosomes are called also *aneuploids* or *heteroploids*.

Drosophila melanogaster has, we know, a pair of X chromosomes, two pairs of large V-shaped ones (second and third), and a pair of small dotlike (fourth) chromosomes in its normal diploid complement (Fig. 69). Bridges found that trisomics with three and monosomics with only one of the large V-shaped autosomes are always lethal; but those with three or with one of the dotlike fourth chromosomes survive (Figs. 80 and 81). The monosomics (referred to in the literature on *Drosophila* genetics as haplo-IV)

are small flies with slender bristles and other minor abnormalities; the trisomics (triplo-IV) differ from normal flies only slightly, one of their peculiarities being rather coarse bristles.

A character called eyeless (eyes small or missing) in *Drosophila melanogaster* is normally inherited as a recessive, giving if crossed to the normal type, segregation of $\frac{3}{4}$ normal: $\frac{1}{4}$ eyeless in the F_2 . But when eyeless flies are outcrossed to haplo-IV, the F_1 generation consists of normal and of eyeless haplo-IV flies. This result shows that eyeless is carried in the dotlike fourth chromosome. Indeed, the single fourth chromosome of the haplo-IV flies in the offspring of the eyeless haplo-IV cross comes evidently from the eyeless parent; since a haplo-IV fly has only one fourth chromosome, the recessive gene eyeless unopposed by its dominant normal allele makes the flies eyeless.

If eyeless is outcrossed to triplo-IV, none of the F_1 flies are eyeless, although about half of them are triplo-IV and the other half diplo-IV. If a triplo-IV fly from this F_1 is test-crossed to eyeless flies, the progeny consists of normal and eyeless in a ratio approaching $\frac{5}{6}$: $\frac{1}{6}$. If the F_1 triplo-IV flies are intercrossed, the F_2 should contain about $\frac{35}{36}$ normal: $\frac{1}{36}$ eyeless. These so-called *trisomic ratios* are explicable if the eyeless gene is carried in the fourth chromosome. The origin of the trisomic ratios is illustrated in Fig. 82. A triplo-IV fly heterozygous for the eyeless gene produces six kinds of gametes, only one of which carries eyeless but no wild-type chromosome. Since two eyeless genes are recessive to a single normal allele, only one-sixth of the zygotes in the test cross will show eyeless, while eyeless flies should form only one thirty-sixth of the total in an F_2 generation.

Aneuploids in Jimson Weed and in Other Plants. A complete series of trisomics has been discovered and studied in the Jimson weed, *Datura stramonium*, by Blakeslee, Belling, and their collaborators. The normal Jimson weed has 12 pairs of chromosomes. In breeding experiments, 12 trisomics have arisen, each of which has a typical set of plant characters which deviates from the wild type in numerous specific ways (Fig. 83). Each of these 12 trisomics has an extra chromosome in one of the 12 sets,

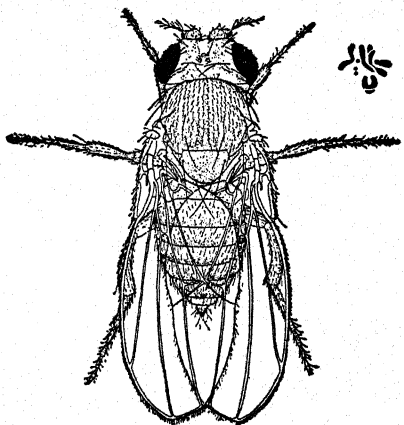


FIG. 81. Triplo-IV individual of *Drosophila melanogaster*. Its chromosome group is at the right. (From Bridges.)

and it is a *different chromosome* which has become doubled in each of the 12 types. Thus the type known as Poinsettia was found always to be associated with an extra chromosome in the set which contains the genes for purple and white flowers.

When any one of these 12 trisomic $2n + 1$ types is crossed with normal, simple Mendelian ratios are not obtained. The complex of characters is transmitted as a group (through the eggs only) to certain of the offspring and does not separate and recombine, as would be expected if the different traits depended on separate genes.

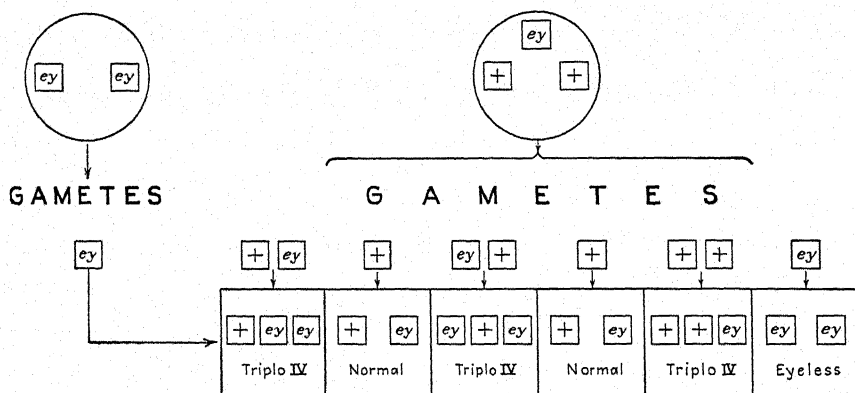


FIG. 82. Trisomic inheritance in the cross of a triplo-IV by a diplo-IV *Drosophila melanogaster*. The diplo-IV individual is homozygous for the recessive gene eyeless (*ey*). The triplo-IV individual has one chromosome with eyeless and two with its normal allele.

It is clear now that the complex of aberrant characters is determined by the extra chromosome, which gives the plant an extra "dose" of all of the genes contained in the duplicated chromosome. The presence of three instead of two of each kind of gene in one chromosome, acting in an individual in which genes in the other chromosomes are merely duplex, produces specific changes in many characters. The irregular transmission of the complex of characters brought about by the extra chromosome is due to the inviability of any pollen which has either more or less than 12 normal chromosomes. Extra chromosomes are thus not transmitted through the pollen but only through the egg cells. Thus the Poinsettia $2n + 1$ form has an extra chromosome in the set which contains the gene pair purple and white. If we let *p* stand for white and *P* for purple, the egg cells of a purple Poinsettia of the genotype *Ppp* have been shown to be in the ratio of $1P:2p$ $2Pp:1pp$, while the only viable pollen grains are

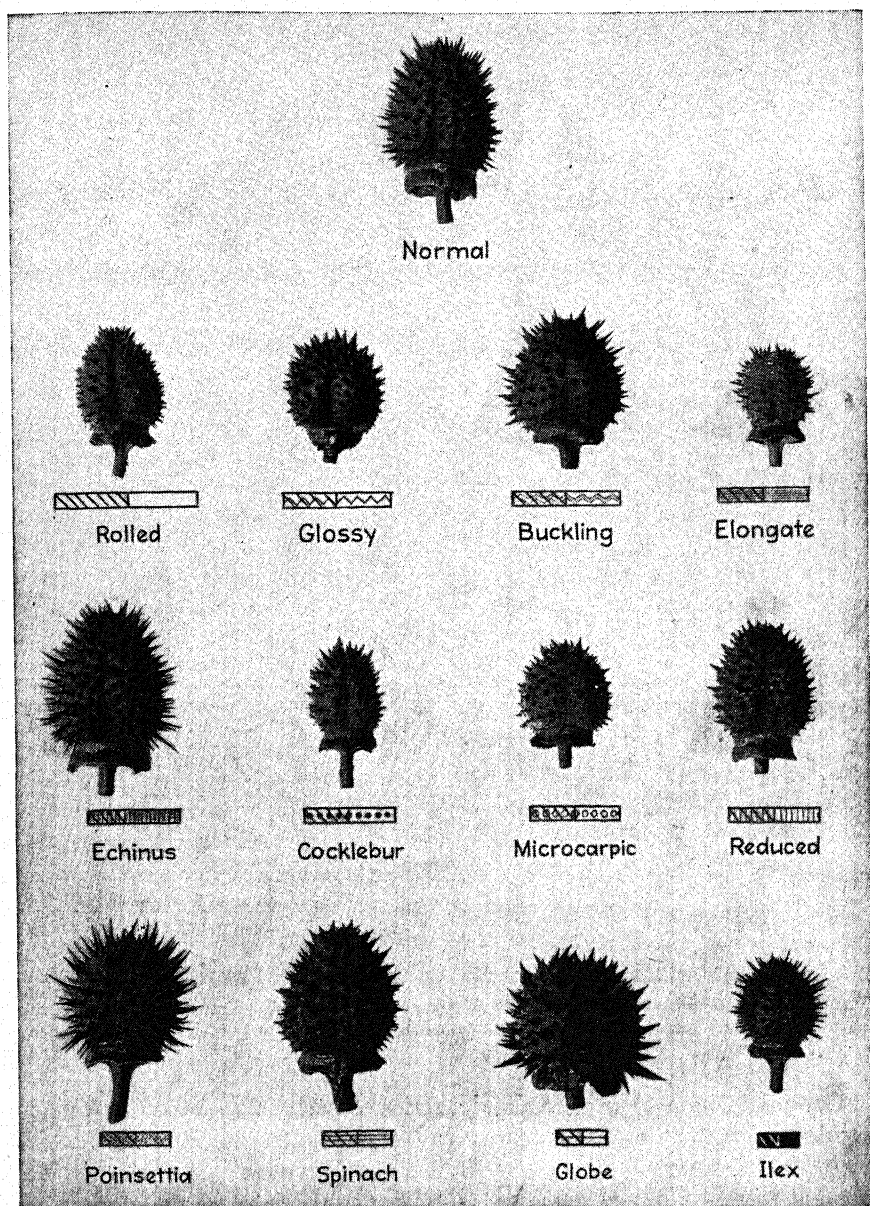


FIG. 83. The seed capsules of primary heteroploid mutants in *Datura*. The extra chromosome in each is shown diagrammatically. (From *Blakeslee*.)

P and p in a ratio of one purple, P , to two white, p . Segregating ratios for purple and white are therefore distorted not only by the triple representation of a gene but by inviability of pollen grains with the extra chromosome. The same is true for trisomic inheritance in maize.

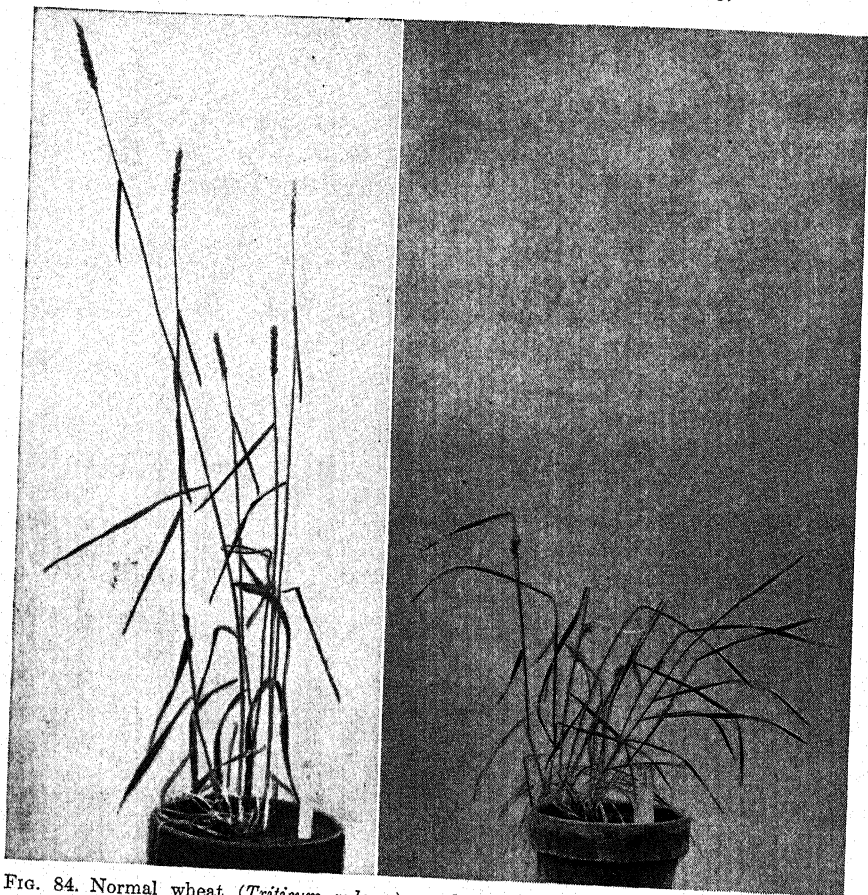


FIG. 84. Normal wheat (*Triticum vulgare*), and a "nullisomic" in which one of the chromosomes (XVI) is missing. Normal on the left, the "nullisomic" on the right. (Courtesy of E. R. Sears.)

One might have expected to find a series of twelve $2n - 1$ types (monosomics) corresponding to the known $2n + 1$ types (trisomics) in the Jimson weed, but the monosomics prove to be mostly inviable. It seems to be a general rule that having some genes present in excess is less deleterious to the organism than having the same genes present in subnormal quantity. In some species, however, both trisomics and monosomics are viable. In

Nicotiana tabacum, 24 different monosomics have been found corresponding to the normal 24 chromosome pairs of this plant. Each of the 24 monosomics is recognizable from the appearance of the plant, although the differences are somewhat less pronounced than those between the 12 trisomics in the Jimson weed. More important, however, is the fact that Clausen and Cameron have analyzed 22 genes known in the tobacco plant by crossing plants carrying them to the various monosomics and observing the manifestation of these genes in the progeny as described above for the eyeless gene in the haplo-IV *Drosophila* (p. 193). They succeeded by this method in associating 18 of these 22 genes with 9 different chromosomes; the location of the 4 remaining genes is unknown since not all the tests have as yet been made.

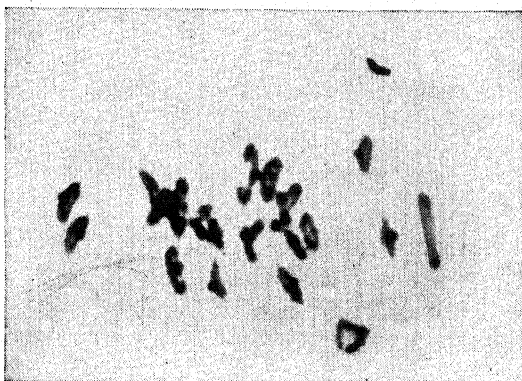


FIG. 85. Metaphase of the last meiotic division in the sixteenth "monosomic" in wheat, *Triticum vulgare*. The unpaired sixteenth chromosome, lacking a partner with which to pair, lies in the upper right corner of the figure. (Courtesy of E. R. Sears.)

In the common wheat, *Triticum vulgare*, with 21 pairs of chromosomes, Sears (1944) obtained 17 of the 21 possible nullisomics. Nullisomics are plants in which a certain chromosome pair is wholly absent (Fig. 84). They should arise in the offspring of two monosomics (Fig. 85), but in most organisms nullisomics are inviable. Trisomics also occur in wheat, but they are difficult to identify, because their external appearance differs little from that of normal plants. With the aid of the nullisomics and monosomics it has been possible to associate 11 different genes with 7 chromosomes.

In addition to these facts, which can be explained only by assuming that genes are parts of chromosomes, many other observations showing how the genes are disposed in the chromosomes, as discussed in the next chapter, lead to the same conclusion.

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PROBLEMS

210. What effect on the sex ratio would a recessive sex-linked lethal factor have in the domestic fowl?

211. What effect on the sex ratio would a recessive sex-linked lethal factor have in man?

Note. In all problems involving sex-linked characters, state results for the two sexes separately.

212. In *Drosophila*, if a white-eyed female is crossed with a red-eyed male and if an F_1 female from this cross is mated with her father and an F_1 male with his mother, what will be the appearance of the offspring of these last two crosses as to eye color?

213. In *Drosophila*, if a homozygous red-eyed female is crossed with a white-eyed male and if an F_1 female from this cross is mated with her father and an F_1 male with his mother, what will be the appearance of the offspring of these last two crosses as to eye color?

214. In *Drosophila*, if a white-eyed female is crossed with a red-eyed male and the F_2 allowed to interbreed freely, what will be the appearance of the F_2 as to eye color?

215. In *Drosophila*, if a homozygous red-eyed female is crossed with a white-eyed male and the F_2 allowed to interbreed freely, what will be the appearance of the F_3 as to eye color?

216. In *Drosophila*, vestigial wings, v , are recessive to the normal long wings, V , and the gene for this trait is not in the sex chromosome. If a homozygous white, long female is crossed with a homozygous red, vestigial male, what will be the appearance of the F_1 ? of the F_2 ? of the offspring of a cross of the F_1 with each parent type?

217. In *Drosophila*, what will be the appearance of the offspring of the following crosses: $Ww Vv \times ww vv$; $ww Vv \times W Vv$?

218. In *Drosophila*, two red-eyed, long-winged flies when bred together produce the following offspring:

Females: three-fourths red, long; one-fourth red, vestigial.

Males: three-eighths red, long; three-eighths white, long; one-eighth red, vestigial; one-eighth white, vestigial.

What are the genotypes of the parents?

219. In *Drosophila*, a cross between Bar-eyed females and wild-type (round-eyed) males produces only Bar-eyed males and females in the F_1 . Wild-type female \times Bar-eyed males produced Bar-eyed females and wild-type males. Explain the inheritance of Bar eye, and predict the appearance of the F_2 from each of these crosses.

220. A girl of normal vision whose father was color-blind marries a man of normal vision whose father was also color-blind. What type of vision will be expected in their offspring?

221. A color-blind man marries a woman of normal vision. They have sons and daughters, all of normal vision and all of whom marry normal persons. Where among the grandchildren may color-blindness be expected to appear? If there are cousin marriages among these grandchildren, where among *their* offspring may color-blindness be expected to appear?

222. A man and woman, both of normal vision, have (1) a color-blind son who has a daughter of normal vision; (2) a daughter of normal vision who has one color-blind and one normal son; and (3) another daughter of normal vision who has five sons, all normal. What are the probable genotypes of grandparents, children, and grandchildren?

223. A man's maternal grandmother had normal vision; his maternal grandfather was color-blind; his mother is color-blind; his father is of normal vision. What are the genotypes, as to vision, of the two parents and grandparents mentioned? What type of vision has this man himself? What type have his sisters? If he should marry a woman genotypically like one of his sisters, what type of vision would be expected in the offspring?

224a. If mating is at random and red-green color-blindness does not affect survival or fertility, what should be the proportion of color-blind women in a population in which 8 per cent of the men are color-blind?

224b. List some of the possible causes of discrepancy between the proportion as calculated above and the proportion actually found.

224c. Assuming an initial frequency of .5 per cent of hemophilia among males at birth and that no male with hemophilia lives to transmit the gene, what should be the frequency of hemophilia after 1, 2, ... , 5 generations of random mating?

Note. In the following problem assume that right-handedness is dominant over left-handedness and brown eye color over blue:

225. The mother of a right-handed, brown-eyed woman of normal vision is right-handed, blue-eyed, and of normal vision, and her father is left-handed, brown-eyed, and color-blind. This woman marries a man who is left-handed, brown-eyed, and of normal vision, and whose father was blue-eyed. What chance will the sons of this couple have of resembling their father phenotypically?

226. In poultry, if a nonbarred cock is crossed with a barred hen and an F_1 female from this cross is mated with her father and an F_1 male with his mother, what will be the appearance of the offspring of these last two crosses, as to barring?

227. In poultry, if a nonbarred cock is crossed with a barred hen and an F_2 from this cross is allowed to interbreed freely, what will be the appearance of the F_3 as to barring?

228. A single-comb, barred cock crossed with a walnut-comb, barred hen produces the following offspring:

4 rose, barred males.	3 rose, nonbarred females.
5 walnut, barred males.	2 walnut, barred females.
2 rose, barred females.	2 walnut, nonbarred females.

What are the genotypes of the parents?

229. In *Drosophila* vermilion eye color is recessive and sex linked. In exceptional cases vermilion female \times normal male produces, in addition to the usual vermilion male and red-eyed female, a few vermilion females and red males. Explain this result, and predict what classes of offspring should appear when the vermilion F_1 females from the above are crossed with red-eyed males.

230. In the fish *Aplocheilus* the wild form is brown; other varieties are blue, red, and white. Sex determination is of the XY type (male heterogamety) as in *Drosophila*. The following results of crossing these varieties were obtained by Aida:¹

Cross 1			
P ₁ White ♀	×	Red ♂	
F ₁ All red			
F ₂ Red ♀	White ♀	Red ♂	White ♂
41	43	67	0
Cross 2			
White ♀ \times F ₁ Red ♂ (from cross 1)			
Red ♀	White ♀	Red ♂	White ♂
0	197	251	0

¹ In the second cross three exceptional individuals have been omitted.

<i>Cross 3</i>			
P ₁ Red ♀	×	White ♂	
F ₁ All red			
F ₂ Red ♀	White ♀	Red ♂	White ♂
87	0	42	33

What is the method of inheritance of red and white color? Draw up a factorial chart to make your explanation clear, and compare the actual numbers in each class with the numbers to be expected on your hypothesis.

In *Aplocheilus* these further results were obtained by Aida. Sex in these fishes cannot be distinguished until they are a year old, so that sex distribution can be given only for those which live to this age:

	P ₁ White ♀ × Brown ♂							
	F ₁ All brown							
F ₂	Brown		Blue		Red		White	
Total young.....	248		57		53		21	
Sex of survivors.....	♀	♂	♀	♂	♀	♂	♀	♂
	77	147	56	0	9	37	19	0

Explain the inheritance and the genetic relations of brown, blue, red, and white. Make a factorial chart as in the previous problem, comparing the actual numbers in each class with the numbers expected on your hypothesis.

231. A factor *l* in *Drosophila* is recessive, lethal, and sex-linked. If female *Ll* is crossed with a normal male, what should be the sex ratio of the progeny?

232. In fowls a factor *K* is recessive and sex linked. All zygotes pure for *K* die before hatching. A male heterozygous for this factor is crossed with normal females and produces 120 live chickens. How many of these would you expect to be males and how many females?

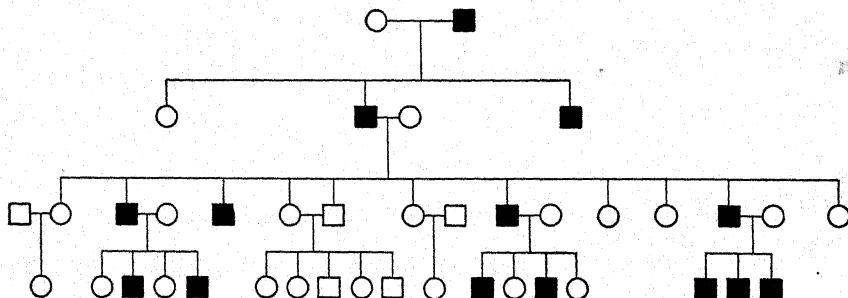
Note. In *Datura* Blakeslee has found 12 mutants, each of which is due to the presence of three chromosomes instead of two, in one of the 12 sets. The mutant Poinsettia he finds to be due to the presence of three chromosomes in the set which carries the genes for purple and white flower color. Letting *A* stand for purple and *a* for white, there may thus be four kinds of Poinsettia plants: *AAA*, *AAa* *Aaa* (purple), and *aaa* (white); and three kinds of normals, *AA*, *Aa* (purple), and *aa* (white). The formation of female gametes here takes place much as it did in the nondisjunctional females of *Drosophila*. In the pollen, however, *none of the grains with the extra chromosome are able to function*, apparently on account of the upset balance between the chromosome sets. All pollen grains formed by purple Poinsettia plants are *A* and *a*, while the eggs may be *A*, *AA*, *Aa*, *a*, or *aa*. The female gametes formed by an individual with the genotype *AAa*, for example, are 2 *A*, 2 *Aa*, 2 *AA*, and 1 *a*. This may perhaps be worked out most readily by writing the genotype thus, $\frac{A}{a} \times \frac{A}{a}$, and making the three possible reduction divisions, *AA* and *a*, *Aa* and *A*, and *Aa* and *A*. If these were male gametes, the *AA* and *Aa* types would not develop, and the survivors would be $\frac{2}{3}$ *A* and $\frac{1}{3}$ *a*.

233. What will be the ratio of purple-flowered to white-flowered plants in the normal ($2n$) offspring and in the Poinsettia ($2n + 1$) offspring from the following crosses:

Female parent \times male parent		Female parent \times male parent	
<i>AAa</i>	<i>AAA</i>	<i>Aaa</i>	<i>AAA</i>
<i>AAa</i>	<i>AAa</i>	<i>Aaa</i>	<i>AAa</i>
<i>AAa</i>	<i>Aaa</i>	<i>Aaa</i>	<i>Aaa</i>
<i>AAa</i>	<i>aaa</i>	<i>Aaa</i>	<i>aaa</i>
<i>AAa</i>	<i>AA</i>	<i>Aaa</i>	<i>AA</i>
<i>AAa</i>	<i>Aa</i>	<i>Aaa</i>	<i>Aa</i>
<i>AAa</i>	<i>aa</i>	<i>Aaa</i>	<i>aa</i>

234. Explain how it is possible, by means of trisomic ratios, to determine the pair of chromosomes associated with a given linkage group.

235. The following is a pedigree of webbed toes in an American family (Schofield 1922).



Propose a hypothesis to account for the transmission of the trait, and calculate the probability that its appearance in males only is due to chance.

236. If the F_1 of a cross between purple-flowered tetraploid and white-flowered tetraploid *Datura* ($PPpp$) is crossed back to its white-flowered parent, what will be the appearance of the offspring?

237. If a tetraploid with the genotype $Pppp$ is selfed, what will be the appearance of its offspring as to flower color?

238. The offspring of haplo-IV, normal-eyed *Drosophila* crossed to diplo-IV, eyeless ones are half normal diplo-IV and half eyeless haplo-IV. If these two F_1 types are crossed, what will their offspring look like as to eyes and number of IV chromosomes?

239. Assume that a triplo-IV, normal-eyed *Drosophila* is crossed with a diplo-IV, eyeless. If the F_1 triplo-IV flies are crossed with the F_1 diplo-IV ones, what will be the eye character and IV-chromosome constitution of their offspring?

CHAPTER IX

LINKAGE AND LINKAGE MAPS

Mendel's principle of independent assortment, as we have seen in the preceding chapters, applies both to genes and to chromosomes. The physical basis of this principle is that the maternal and paternal members of each pair of chromosomes are distributed independently to the gametes at meiosis. It is for this reason that genes carried in different chromosomes undergo independent assortment and produce the ratios of differentiating characters which Mendel discovered and explained so successfully.

It must be evident, however, that the number of genes in any organism, which may be reckoned in the thousands, exceeds the number of pairs of chromosomes, which seldom reach 100. In *Drosophila*, for example, hundreds of genes have already been studied, yet there are only 4 pairs of chromosomes. If all genes are in chromosomes, it follows that each chromosome must contain many genes and that genes in the same chromosome will not be assorted independently. We may be prepared then to find that Mendel's second law is not universal but is limited to genes in *different* chromosomes.

Indeed, an exception to this law was discovered not long after Mendel's work was rediscovered, for in 1906 Bateson and Punnett found two pairs of alleles in sweet peas which did not assort independently. Instead, when two alleles, such as *A* and *B*, came from the same parent ($AA\ BB \times aa\ bb$), they tended to enter the same gamete and to be transmitted together, and when the same alleles came from different parents ($AA\ bb \times aa\ BB$) they tended to enter different gametes and to remain apart. The first peculiarity was called *coupling* and the second *repulsion*.

Linkage. No satisfactory explanation of these exceptions was reached until Morgan in 1910 found similar exceptions in *Drosophila* and saw that coupling and repulsion are but two aspects of a single phenomenon which he called *linkage*. He supposed that this tendency of linked genes to remain in their original combinations was due to their residence in the same chromosome. Furthermore, Morgan advanced the idea that the degree or strength of linkage depends upon the distance between the linked genes in the chromosome. This proved to be a very fruitful idea, for it soon developed into the theory of the linear arrangement of genes in the

chromosomes and has served as the basis for the construction of genetical or linkage maps of chromosomes.

Linkage in Maize. The phenomena of linkage are particularly clear in maize, in which the recombination between linked genes is similar in both sexes and in which traits visible in the seeds can be easily observed in large populations, the same ear having several hundred seeds. A good example is provided by the results of Hutchison, who crossed a variety of maize having seeds which were colored and normally filled out (full) to one with colorless and shrunken seeds. In other experiments it had been shown that color, gene *C*, was a simple dominant to colorless, *c*, while normal or full endosperm, gene *S*, is dominant to shrunken, *s*. The parents accord-

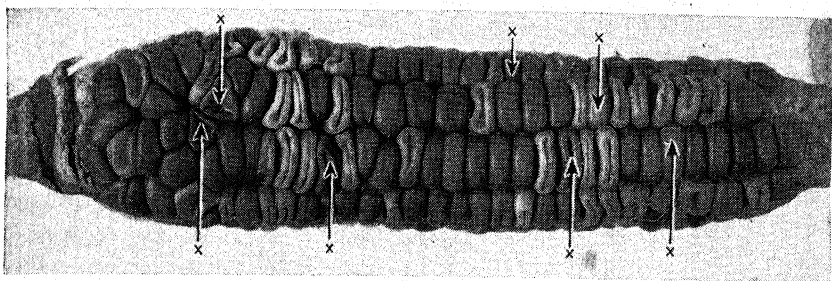


FIG. 86. The kernels on this ear show linkage of factors for aleurone color and for shrunken endosperm. It resulted from the cross of a heterozygous plant (which had received the genes for *colored* and *full* from one parent and for *colorless* and *shrunken* from the other) with a double recessive colorless, shrunken plant. Most of the kernels are colored and full, or colorless and shrunken (parental combinations), and a few have the new combinations *colored shrunken* and *colorless full*. Such new combinations are designated by *x*. (From Hutchison, in *Journal of Heredity*.)

ingly were *CC SS* and *cc ss*, and the F_1 , as expected, had colored, full seeds which must have the genotype *CS/cs*.¹

If *C* and *S* assort independently, in accordance with Mendel's second principle, these F_1 plants should produce four types of gametes *CS*, *Cs*, *cS*, and *cs* in equal numbers. The easiest way to test this gametic ratio is to make a test cross of F_1 to the double recessive *cc ss* which, on the above expectation, would yield four classes of progeny in the ratio 1:1:1:1. When the cross was made, however, this expectation was not realized, but the following result was obtained (data from Hutchison 1922):

P_1 Colored, full \times colorless, shrunken
 CS/CS | cs/cs
 F_1 Colored, full

¹ In representing genes known to be linked, the gene combinations are written as they enter the zygote, those from one parent above a line, those from the other below it.

F ₁ Colored, full × colorless, shrunken				
	CS/cs		cs/cs	
	Backcross Progeny			
Colored, Full CS/cs	Colored, Shrunken Cs/cs	Colorless, Full cS/cs	Colorless, Shrunken cs/cs	Total
4,032	149	152	4,035	8,368

The colored, full and colorless, shrunken seeds are *more* frequent and the colored, shrunken and colorless, full seeds are less frequent than expected (Fig. 86). Now, the parents had colored, full and colorless, shrunken seeds. These are *parental combinations* of characters, while in the *recombinations* the associations of the characters have changed. In independent assortment the *parental combinations*, CS and cs , and recom-

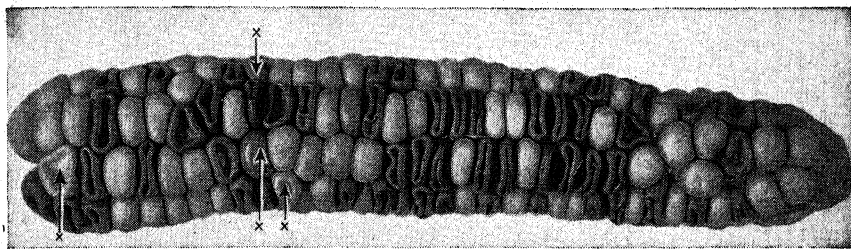


FIG. 87. An ear of similar ancestry to that in Fig. 86 except that the genes entered the F₁ plants in different combinations, *colored* and *shrunken* coming together from one pure parent and *colorless* and *full* from the other. The recombinations (x) in this case are *colored full* and *colorless shrunken*. (From Hutchison, in *Journal of Heredity*.)

binations, Cs and cS , would be equally frequent. But in the experiment shown the gametic combinations must have been as follows:

Parental combinations.....	CS 4,032
	cs 4,035
	8,067, or about 96.4 per cent of the total gametes tested
Recombinations.....	Cs 149
	cS 152
	301, or about 3.6 per cent of the total gametes tested

It is obvious that the two pairs of genes $C-c$ and $S-s$ have not assorted independently. The *parental combinations* greatly exceed the expected 50 per cent; they remain combined or linked in 96.4 per cent and are recombined in only 3.6 per cent of the gametes.

When another experiment was so arranged that the same genes entered the cross in different associations, that is, when parents with colorless, full

seeds were crossed with those with colored, shrunken seeds, it was found that again the parental combinations were in excess, although now these parental combinations are just the opposite of what they were in the first experiment (Fig. 87). The results of the second experiment were as follows:

P ₁ Colorless, full × colored, shrunken				
	cS/cS		Cs/Cs	
F ₁ Colored, full				
F ₁ Colored, full × colorless, shrunken				
	cS/Cs		cs/cs	
Backcross Progeny				
Colored, Full CS/cs 638	Colored, Shrunken Cs/cs 21,379	Colorless, Full cS/cs 21,906	Colorless, Shrunken cs/cs 672	Total 44,595

Here the parental combinations are $21,379 + 21,906 = 43,285$, or 97.06 per cent of all, while the recombinations are $638 + 672 = 1,310$ or 2.94 per cent of the total. The ratio between parental combinations and recombinations is only slightly lower than it was in the first experiment. It is obvious that, whatever the parental combinations of two different pairs of linked genes may be, linkage tends to keep them together in about the same proportion of the gametes of the double heterozygote.

✓ **Crossing Over.** We have seen that, in the experiments of Hutchison, gametes with the parental combinations of genes were always more numerous than gametes containing gene recombinations. This is generally observed in experiments on linkage in both plants and in animals. Furthermore, the frequency of recombination, and consequently the intensity of linkage, is more or less constant for any pair of linked genes (see, however, p. 224) but may be very different for different genes.

According to the theory first proposed by T. H. Morgan, linkage is caused by linked genes being carried in the same chromosome. If, however, the chromosomes remain intact in inheritance, then two genes located in the same chromosome should remain together in all cases; in other words, linkage should be complete. This is not what actually happens, for linkage is normally only partial, and the linked genes sometimes separate. Thus, the genes for seed color, *C*, and for full or shrunken endosperm *S*, in maize remain associated in parental combinations in about 97 per cent of gametes but break apart in about 3 per cent (see above). ✓ T. H. Morgan ascribed the recombination of linked genes to interchange of parts between homologous chromosomes, which he called *crossing over*. The behavior of the chromosomes in the cross of maize with colored, full and with colorless, shrunken seeds can, then, be represented as shown in the diagram in Fig. 88. Crossing over takes place in the segment of the chromosome between the locations of the genes *C* and *S* in some cells but not

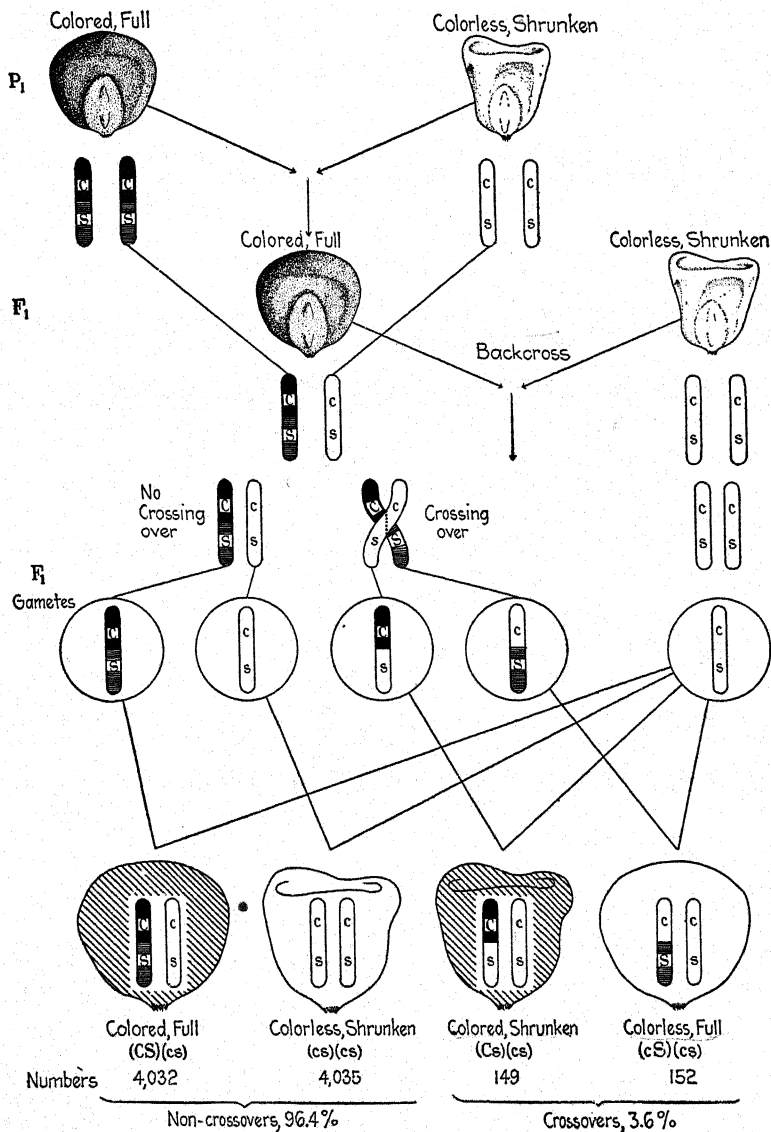


FIG. 88. Diagram showing the chromosome explanation of linkage and crossing over in maize. The history of the genes for colored-colorless ($C-c$ and full-shrunken ($S-s$), and of the chromosomes in which they are located, is traced through a cross between two pure types and the backcross of F₁ with a double recessive. (Data from Hutchison.)

in others, so that about 97 per cent of the gametes contain the parental gene combinations and 3 per cent contain recombinations.

Evidence bearing on the mechanism of crossing over has been obtained from a study of the behavior of chromosomes at meiosis. It has been pointed out in Chapter VII that, during the meiotic prophase, homologous

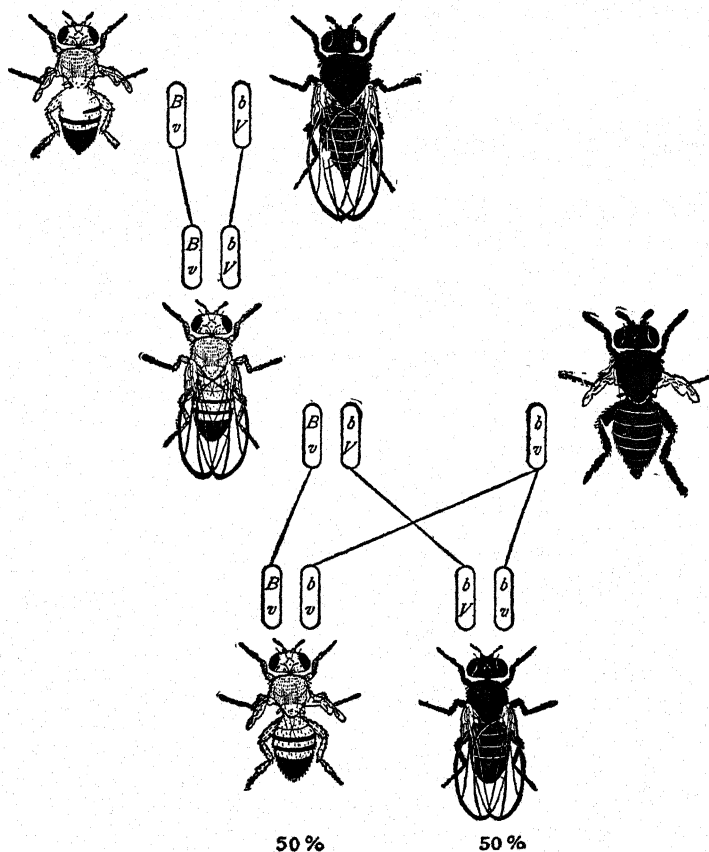


FIG. 89. Complete linkage (no crossing over) in the male *Drosophila*. Results of a cross between a gray, vestigial male and a black, long female; and of a backcross of the F_1 male with a black, vestigial female. Note that the offspring of this backcross are all like the original parents and that there are no crossovers. (From Morgan, Sturtevant, Muller, and Bridges, courtesy of Henry Holt & Company.)

(maternal and paternal) chromosomes come together and pair by becoming apposed to each other throughout their length. There is evidence that this pairing is brought about by mutual attraction of similar parts of the homologous chromosomes, which presumably contain allelic genes. Now, during the transition between the pachytene and the diplotene stages, the

paired chromosomes divide each into two chromatids, so that the bivalent is now composed of four chromatids. At about the same time when the chromosomes are first seen to be divided, the chromatids establish one or

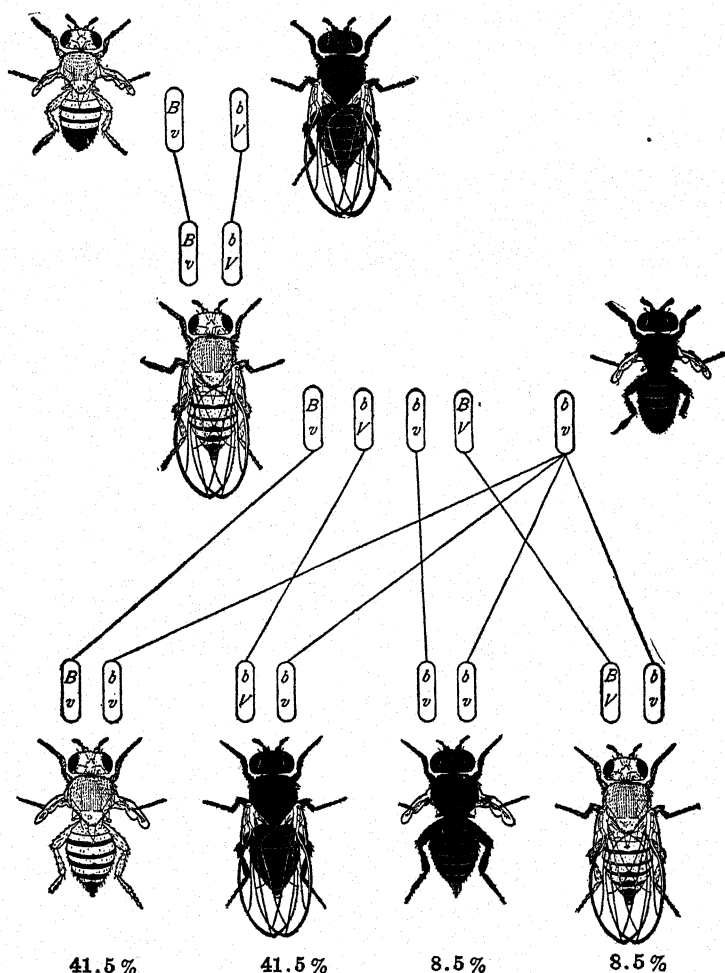


FIG. 90. Crossing over in the female of *Drosophila*. Results of a cross between a gray, vestigial male and a black, long female; and of a backcross of the F₁ female with a black, vestigial male. (From Morgan, Sturtevant, Muller, and Bridges, courtesy of Henry Holt & Company.)

more exchanges, or chiasmata, per bivalent (Fig. 60, p. 162). At each chiasma, two of the four chromatids have become broken and then re-joined, so that the new chromatids are compounded of sections of the original ones (Fig. 94). This process, first correctly understood by Jans-

sens in 1909, has been later studied, especially by Darlington and his colleagues. The details of this process are still by no means clear; it remains, for example, unknown just what force causes the breakage of the chromatids and the establishment of the chiasmata. Two facts which have important genetic connotations seem, however, to be well established. First the process of crossing over is a remarkably precise one, so that the two chromatids at a chiasma exchange exactly equivalent segments, neither of the chromatids gaining or losing genes. Second every chiasma visible under a microscope corresponds to a crossover and to a genetically perceptible recombination of genes linked in the chromosome.

Linkage in *Drosophila* Males and Females. In most of the organisms which have furnished the classical materials for genetic studies, such as maize, peas, poultry, mice, man, and others, recombination of linked genes takes place both in females and in males. Similarly, in most organisms which are favorable for cytological studies, formation of chiasmata is observed in both female and male meiosis. The most thorough and extensive studies on linkage and crossing over have, however, been made on species of *Drosophila*, and it happens that in these insects the situation is very different in the two sexes. In *Drosophila*, crossing over rarely or never takes place in the male.

If a gray-bodied, vestigial-winged fly is crossed to a black-bodied, long-winged one, the F_1 generation consists of gray, long-winged (normal) flies (Figs. 89 and 90). The gene for gray body color, B , is, hence, dominant over its allele, which causes black body color, b , and the gene for long wings, V , is dominant over its vestigial allele, v . Now, if the F_1 male hybrids are crossed to double recessive females (black-bodied, vestigial-winged females), only two kinds of offspring are produced—gray, vestigial and black, long (Fig. 89). The expected types of crossovers—gray, long and black, vestigial—do not appear at all. If, however, an F_1 female fly is crossed with a black, vestigial male, the four expected types (Fig. 90) are produced in the following proportions (data from Morgan, 1919):

Noncrossovers		Crossovers	
Gray, vestigial	Black, long	Black, vestigial	Gray, long
41.5 per cent	41.5 per cent	8.5 per cent	8.5 per cent
83 per cent		17 per cent	

Crossing over is evident in about 17 per cent of the gametes. The second experiment shows that a perceptible distance separates the genes for black and vestigial and that absence of crossovers in the gametes of the male is not due to the extreme closeness of the genes in the chromosome. It must be due, then, to conditions peculiar to the male, such as the nonoccurrence of chiasmata in spermatogenesis.

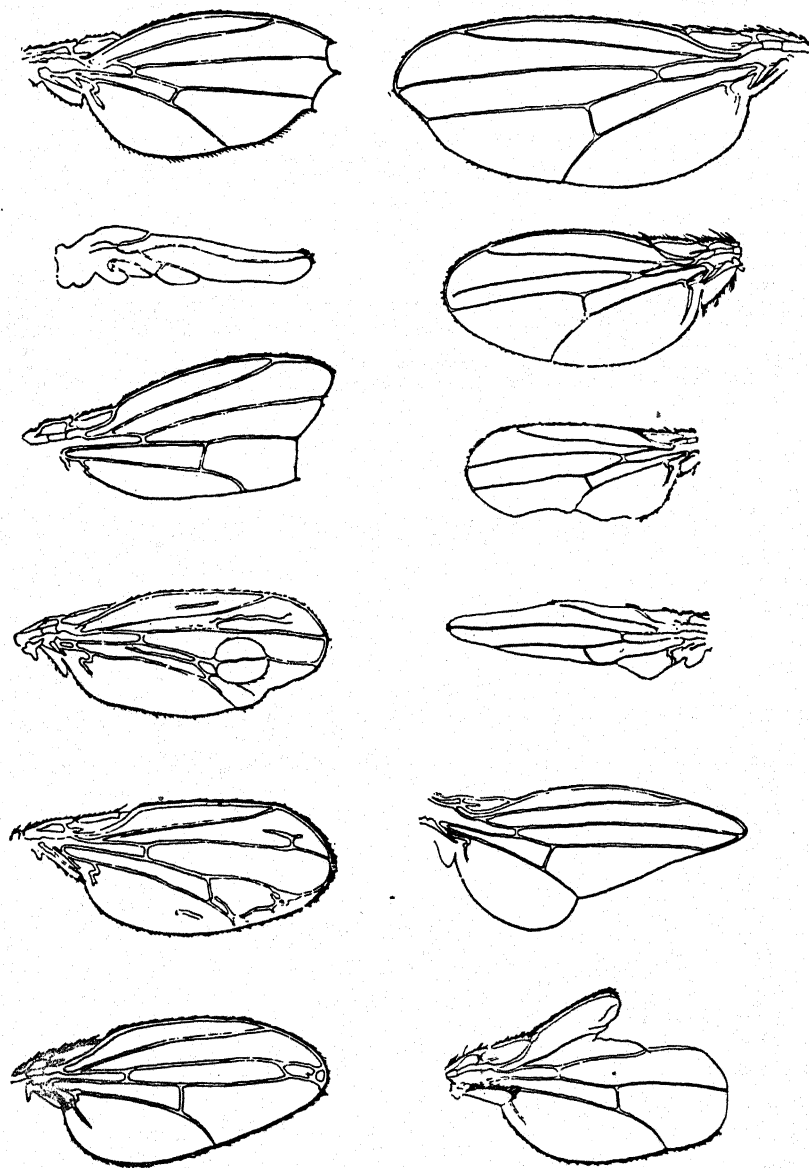


FIG. 91

FIG. 92

FIG. 91 and 92. Mutants of *Drosophila melanogaster* showing changes in the size, shape, or venation of the wing. Each wing is characteristic of one mutant type. (From Bridges and Brehme.)

Only a few cases are known in which linkage is complete in one sex and crossing over occurs more or less freely in the other. In all species of *Drosophila* so far studied in this respect, crossing over is absent or exceedingly rare in males, while in the silkworm moth it seems to be absent in females. Cytological study of spermatogenesis in male *Drosophila* dis-

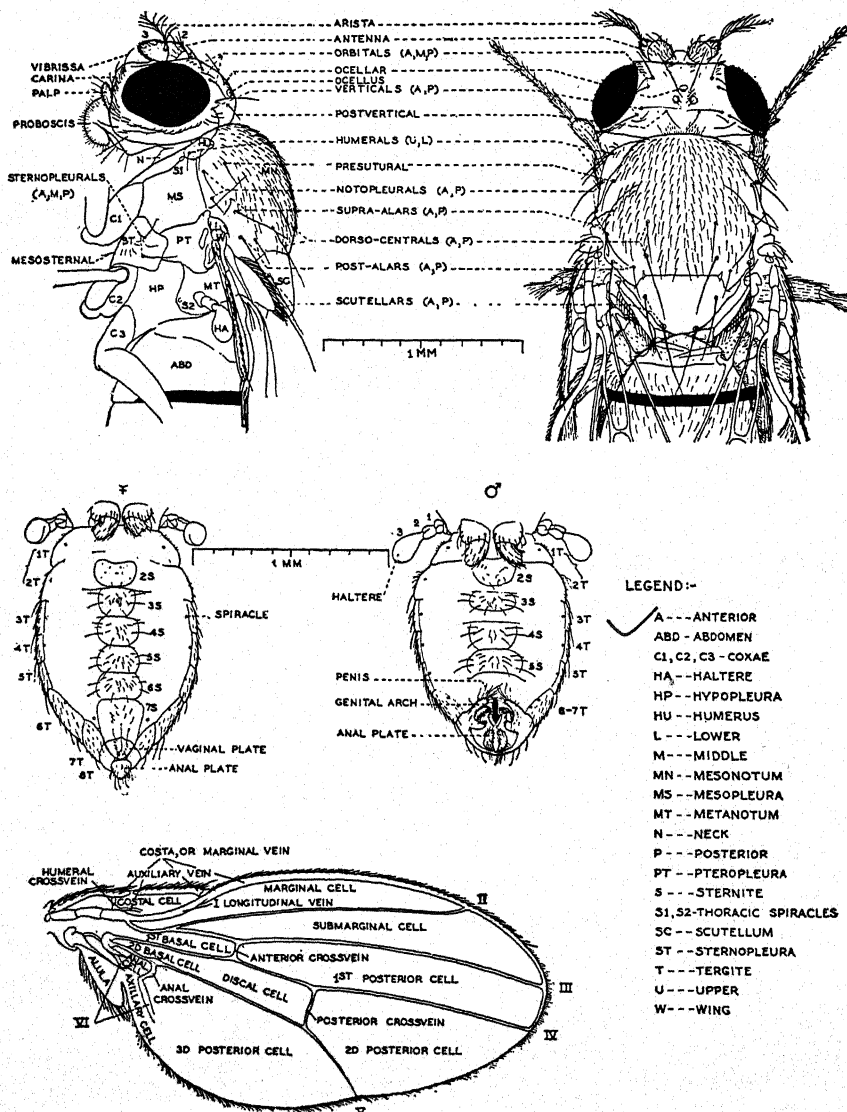


FIG. 93. Details of the external structure of *Drosophila melanogaster*. (From Bridges.)

closes that the homologous chromosomes undergo pairing in the spermatocytes but that no chiasmata are established, at least not in the autosomal bivalents. The bivalents eventually come to consist of four chromatids lying parallel to each other; at the first meiotic division, pairs of chromatids go to each pole, and at the second meiotic division, single chromatids pass to each cell, which is transformed into a spermatozoon. Female meiosis in *Drosophila* is unfavorable for detailed cytological study, but it appears that the bivalents do have chiasmata.

Linkage Groups and Chromosomes. If a certain gene, *A*, is linked to two other genes, *B* and *C*, these latter genes are also linked with each other. If several genes are known in an animal or plant species, crosses may be arranged in which either independent assortment or linkage of pairs or of groups of several pairs of genes can be observed. Such experiments are particularly easy in species of *Drosophila*, because the absence of crossing over in the male hybrids shows at once which genes are linked and which segregate independently. The genes known in a species may thus be arranged in a number of *linkage groups* the members of which show linkage with each other. In genetically well studied species, the number of linkage groups is found to be equal to the number of chromosome pairs (the haploid number of chromosomes) which the species possesses.

Favorite materials for studies on linkage and kindred problems are the vinegar flies, *Drosophila melanogaster*, and related species. They breed easily under laboratory conditions, their culture is simple and inexpensive, their life cycle is only about two weeks long under proper conditions, the fecundity is so high that a pair of parents may produce hundreds and even thousands of offspring, they produce numerous hereditary variants (mutations), and their chromosomes, especially in the cells of larval salivary glands, are uncommonly favorable for observation. Thousands of mutations have been observed in *D. melanogaster* since about 1909, when this species was first used for such studies by T. H. Morgan and his collaborators. Examples of some of these mutant types are shown in Figs. 91 and 92. Drawings of the chief external details of the normal, or wild-type, fly are shown in Fig. 93. Studies on the inheritance of these mutant types have shown that they belong to four linkage groups, some of the members of which are listed in Table XXI.

The numbers of main gene loci belonging to the four linkage groups in *Drosophila melanogaster* are, according to Bridges and Brehme (1944), as follows: first, 141; second, 228; third, 156; fourth, 12. The species possesses, as we know, four pairs of chromosomes (in other words, the haploid chromosome number is $n = 4$) (see Fig. 69, p. 177). The rod-shaped X chromosome is present twice in females and only once in males, its partner in the male being the hook-shaped Y chromosome. Since the genes of

TABLE XXI. A LIST OF GENES MOST USEFUL IN GENETIC EXPERIMENTS IN THE FOUR LINKAGE GROUPS CORRESPONDING TO THE FOUR CHROMOSOMES OF *Drosophila melanogaster* (After Bridges and Brehme, 1944)

Symbols and locations correspond to those in Fig. 98, p. 227.

Capital letters indicate dominant mutations.

Chromosome X or I	Chromosome II	Chromosome III	Chromosome IV
yellow body (<i>y</i> , 0.0)	net veins (<i>net</i> , 0.0)	roughoid eyes (<i>ru</i> , 0.0)	shaven bristles (<i>sv</i> , 0.0)
scute bristles (<i>sc</i> , 0.0)	aristaless (<i>al</i> , 0.0)	veinlet wing (<i>ve</i> , 0.2)	cubitus interruptus venation (<i>ci</i> , 0.0)
silver body (<i>svr</i> , 0.0)	ochracea eyes (<i>ocr</i> , 0.0)	Roughened eyes (<i>R</i> , 1.4)	grooveless scutellum (<i>gvl</i> , 0.0)
broad wing (<i>br</i> , 0.6)	Star eyes (<i>S</i> , 1.3)	javelin bristles (<i>ju</i> , 19.2)	eyeless (<i>ey</i> , 0.2)
prune eyes (<i>pn</i> , 0.8)	heldout (<i>ho</i> , 4.0)	sepia eyes (<i>se</i> , 26.0)	
white eyes (<i>w</i> , 1.5)	echinoid eyes (<i>ed</i> , 11.0)	hairy body (<i>h</i> , 26.5)	
facet eyes (<i>fa</i> , 3.0)	fat body (<i>ft</i> , 12.0)	approximated veins (<i>app</i> , 37.5)	
echinus eyes (<i>ec</i> , 5.5)	dumpy wing (<i>dp</i> , 13.0)	Glued eyes (<i>Gl</i> , 41.4)	
bifid veins (<i>bi</i> , 6.9)	clot eyes (<i>cl</i> , 16.5)	thread arista (<i>th</i> , 43.2)	
ruby eyes (<i>rb</i> , 7.5)	Jammed wing (<i>J</i> , 41.0)	scarlet eyes (<i>st</i> , 44.0)	
crossveinless (<i>cv</i> , 13.7)	black (<i>b</i> , 48.5)	clipped wing (<i>cp</i> , 45.3)	
roughex eyes (<i>ru_x</i> , 15.0)	reduced bristles (<i>rd</i> , 51.0)	Wrinkled wing (<i>W</i> , 46.0)	
carmine eyes (<i>cm</i> , 18.9)	purple eyes (<i>pr</i> , 54.5)	inturned bristles (<i>in</i> , 47.0)	
cut wing (<i>ct</i> , 20.0)	Bristle short (<i>Bl</i> , 54.8)	radius incompletus veins (<i>ri</i> , 47.1)	
singed bristles (<i>sn</i> , 21.0)	light eyes (<i>lt</i> , 55.0)	pink eyes (<i>p</i> , 48.0)	
lozenge eyes (<i>lz</i> , 27.7)	straw body (<i>stw</i> , 55.1)	blistery wing (<i>by</i> , 48.7)	
raspberry eyes (<i>ras</i> , 32.8)	tarsi fused (<i>ti</i> , 55.9)	curled wing (<i>cu</i> , 50.0)	
vermilion eyes (<i>v</i> , 33.0)	cinnabar eyes (<i>cn</i> , 57.5)	mussed wing (<i>mu</i> , 50.0)	
miniature wing (<i>m</i> , 36.1)	engrailed (<i>en</i> , 62.0)	karmoisin eyes (<i>kar</i> , 52.0)	
dusky wing (<i>dy</i> , 36.2)	scabrous eyes (<i>sca</i> , 66.7)	Stubble bristles (<i>Sb</i> , 58.2)	
sable body (<i>s</i> , 43.0)	vestigial wing (<i>vg</i> , 67.0)	spineless bristles (<i>ss</i> , 58.5)	
garnet eye (<i>g</i> , 44.4)	Lobe eye (<i>L</i> , 72.0)	Roof wing (<i>Rf</i> , 59.0)	
scalloped wing (<i>sd</i> , 51.5)	curved wing (<i>c</i> , 75.5)	stripe thorax (<i>sr</i> , 62.0)	
uneven eyes (<i>un</i> , 54.4)	plexus veins (<i>px</i> , 100.5)	glasse eyes (<i>gl</i> , 63.1)	
forked bristles (<i>f</i> , 56.7)	brown eyes (<i>bw</i> , 104.5)	Delta veins (<i>Dl</i> , 66.2)	
Bar eyes (<i>B</i> , 57.0)	speck wing (<i>sp</i> , 107.0)	Hairless bristles (<i>H</i> , 69.5)	
Beadex wing (<i>Bx</i> , 59.4)		ebony body (<i>e</i> , 70.7)	
fused veins (<i>fu</i> , 59.5)		Prickly bristles (<i>Pr</i> , 90.0)	
carnation eyes (<i>car</i> , 62.5)		rough eyes (<i>ro</i> , 91.1)	
bobbed bristles (<i>bb</i> , 66.0)		Beaded wing (<i>Bd</i> , 93.8)	
		claret eyes (<i>ca</i> , 100.7)	
		brevis bristles (<i>bv</i> , 104.3)	

the first linkage group all show the sex-linked inheritance discussed in Chapter VIII, they evidently are localized in the X chromosomes. The fourth linkage group contains the fewest genes, which makes it probable that it corresponds to the small dotlike pair of chromosomes (Fig. 69, p. 177). This inference is fully confirmed by the fact that the genes of the fourth linkage group give monosomic and trisomic segregation ratios in crosses with haplo-IV and triplo-IV flies (see p. 179). The second and the third linkage groups correspond, then, to the two large V-shaped chromosome pairs, called the second and third chromosomes.

In species of *Drosophila* other than *Drosophila melanogaster*, the haploid chromosome numbers vary from 3 to 6, and the numbers of linkage groups vary accordingly. In other genetically well-studied organisms a similar correspondence is observed. Thus, maize has 10 pairs of chromosomes, and approximately 400 genes studied in this plant fall into 10 linkage groups, while in peas, *Pisum sativum*, there are 7 linkage groups and 7 chromosomes. As could be expected, in several organisms the number of linkage groups known is less but is nowhere greater than the haploid number of chromosomes. Thus, in the mouse 13 linkage groups are known for 20 chromosomes, in rabbit 11 linkage groups for 22 chromosomes, in the tomato 10 linkage groups for 12 chromosomes, etc.

Linear Arrangement of Genes in the Chromosomes. We have seen that the parental combinations are always more frequent than recombinations of genes among the gametes produced by an individual heterozygous for two linked genes. The frequency of recombination (that is, of the cross-over classes) is, under standardized conditions, constant and characteristic for any pair of linked genes. As first supposed by T. H. Morgan, this frequency is proportional to the distance in the chromosome between the genes in question, that is, the greater the distance, the more likely it is that a crossover will occur within it. A very significant new regularity is revealed if, instead of observing recombination of two linked genes, an experiment is made in which three or more linked genes are involved.

Bridges and Olbrycht crossed *Drosophila melanogaster* flies with rough eyes due to the gene echinus, *ec*, by flies with scute, *sc* (certain bristles missing), and crossveinless, *cv* (absence of the cross veins in the wing). Since these three genes are recessive to the corresponding normal condition and are sex-linked, the hybrid females were normal, or wild type, in phenotype, that is, they had nonrough eyes, and all bristles and cross veins were present. The hybrid females had one X chromosome carrying the gene echinus and the normal alleles of scute and crossveinless, and another X chromosome with a normal allele of echinus and with mutant alleles of scute and of crossveinless. In *Drosophila* genetics it is convenient to denote the normal, or wild-type alleles (that is, the alleles present

in normal wild flies as they are found outside of laboratories) by plus signs; and so the X chromosomes of the hybrid females may be written as follows: $\frac{+ \text{ } ec}{sc \text{ } + cv}$. These females were test-crossed to males carrying the three recessive genes scute, echinus, and crossveinless (*sc ec cv* males). If these genes were not linked, the offspring of the test cross, in accordance with the second law of Mendel, should have contained eight classes of offspring in equal numbers, representing the possible combinations of the three parental genes. In the actual experiment the following results were obtained (Table XXII):

TABLE XXII

Parental Combinations	{ echinus (+ <i>ec</i> +).....	810	1,638
	{ scute crossveinless (<i>sc</i> + <i>cv</i>)	828	
Recombinations	{ scute echinus (<i>sc ec</i> +).....	62	150
	{ crossveinless (+ + <i>cv</i>)	88	
	{ scute (<i>sc</i> + +).....	89	192
	{ echinus crossveinless (+ <i>ec cv</i>)	103	
	wild type (+ + +).....	0	0
	{ scute echinus crossveinless (<i>sc ec cv</i>)	0	
Total.....		1,980	

The parental combinations are far more frequent than any of the recombination classes; some of the latter, namely, wild type and *sc ec cv*, are altogether missing. Let us now compute the frequencies of recombinations between the three genes involved. The genes scute and echinus and their normal alleles entered the cross from different parents, and they remained in different individuals in $1,638 + 192 = 1,830$ flies, which represent, then, the parental combinations of these two genes. But in 62 flies in the test-cross generation the genes scute and echinus are present together, and in 88 flies their normal alleles are present together. These $62 + 88 = 150$ flies represent, then, the recombinations of the genes *sc* and *ec*. The frequency of recombinations of *sc* and *ec* is, accordingly, $(150 \times 100)/1,980 = 7.6$ per cent. Similarly, the genes echinus and crossveinless and their normal alleles entered the cross from different parents. We find, however, 89 flies which have neither *ec* nor *cv* and 103 flies which have both *ec* and *cv*, in other words, $89 + 103 = 192$ recombinations for these genes. The frequency of recombinations between *ec* and *cv* is, then, $(192 \times 100)/1,980 = 9.7$ per cent. Finally, the genes scute and crossveinless and their normal alleles were introduced in the cross by the same parents; in the progeny of the test cross one of these genes is

present without the other in $62 + 88 + 89 + 103 = 342$ flies; the frequency of recombination between *sc* and *cv* is, accordingly, $(342 \times 100)/1,980 = 17.3$ per cent.

To summarize, the frequencies of recombinations between the genes scute, echinus, and crossveinless are

<i>sc-ec</i>	7.6 per cent
<i>ec-cv</i>	9.7 per cent
<i>sc-cv</i>	17.3 per cent

If the frequency of crossing over giving rise to recombination of linked genes is a function of the distance between these genes in the chromosome, then the distance *sc-cv* equals the sum of the *sc-ec* and *ec-cv* distances. In general, if the crossover distances are small and *if the distance between genes a and b and c are, respectively, ab and bc, then the distance between a and c is equal to either $ab + bc$ or $ab - bc$* . Now, in geometry such a relationship results if the points *a*, *b*, and *c* lie on a straight line, and in genetics this relationship is the basis of the theory according to which *genes are arranged in chromosomes in a single linear series*. In the example under consideration, the genes evidently form a series *sc-ec-cv* (or *cv-ec-sc*, which is equivalent), and we may draw a *genetic map* of the part of the chromosome in which these genes are located thus:

<i>sc</i>	<i>ec</i>	<i>cv</i>
7.6	9.7	

Double Crossing Over. In principle, it is a rather simple matter to add to the above map the positions of more and more genes, until all the genes belonging to the same linkage group have been entered. For this purpose, experiments are planned so that at least two previously known loci¹ and one or more new ones are involved in crosses. Then, using the fact that the distance between genes *a* and *c* is either the sum or the difference of the *a-b* and *b-c* distances, it is determined whether the new gene lies between, or to one side of, the old ones.

Crossveinless (*cv*) flies were crossed by Bridges and Olbrycht to flies carrying echinus (*ec*) and cut (notched wing margin, *ct*, a sex-linked recessive gene). The hybrid females, which must have had the genetic

¹ The term *locus* (plural *loci*) is used both to indicate the location of a gene on a chromosome map and also to designate the unit, variants of which act as alleles. In the latter sense, "locus" is almost equivalent to "gene." One speaks, however, of the alleles which determine the red, eosin, and white eye colors in *Drosophila* as "the gene for red," for "eosin," and for "white eye colors," while these three genes together are referred to as variants or alleles of the same "white" locus. The locus is usually named for the first variant allele which is found.

structure $\frac{+ cv +}{ec + ct}$, were crossed to *ec cv ct* males. In the next generation the classes of flies shown in Table XXIII were obtained.

TABLE XXIII

Noncrossovers	$\left\{ \begin{array}{l} \text{crossveinless (+ cv +)} \\ \text{echinus cut (ec + ct)} \end{array} \right.$	$\left. \begin{array}{r} 2,207 \\ 2,125 \end{array} \right\}$	81.5 per cent
Crossovers between <i>ec</i> and <i>cv</i>	$\left\{ \begin{array}{l} \text{echinus crossveinless (ec cv +)} \\ \text{cut (+ + ct)} \end{array} \right.$	$\left. \begin{array}{r} 273 \\ 265 \end{array} \right\}$	10.1 per cent
Crossovers between <i>cv</i> <i>ct</i>	$\left\{ \begin{array}{l} \text{echinus (ec + +)} \\ \text{crossveinless cut (+ cv ct)} \end{array} \right.$	$\left. \begin{array}{r} 217 \\ 223 \end{array} \right\}$	8.3 per cent
Double crossovers	$\left\{ \begin{array}{l} \text{wild type (+ + +)} \\ \text{echinus crossveinless cut (ec cv ct)} \end{array} \right.$	$\left. \begin{array}{r} 5 \\ 3 \end{array} \right\}$	0.15 per cent
Total.....		5,318	

As in the example presented in Table XXII, the analysis of the data in Table XXIII begins by determining which classes represent the parental combinations of each pair of genes and which are recombinations (crossovers) of these genes. It should be noted that the two numerically smallest classes of flies, wild type and echinus crossveinless cut, represent recombinations both of the genes *ec* and *cv* and of *cv* and *ct*; these classes are, however, parental combinations as far as the genes *ec* and *ct* are concerned. The frequency of recombinations between the genes *ec* and *cv* is, therefore, $10.1 + .1 = 10.2$ per cent, between *cv* and *ct* $8.3 + .1 = 8.4$ per cent, and between *ec* and *ct* $10.1 + 8.3 = 18.4$ per cent. From these data the relative positions of their genes may be determined. Since the distance *ec-cv* is 10.2 per cent and the distance *cv-ct* is 8.4 per cent, the distance *ec-ct* may be either $10.2 + 8.4 = 18.6$ per cent or $10.2 - 8.4 = 1.8$ per cent. The former figure would mean that the gene order is *ec-cv-ct* and the latter that it is *ec-ct-cv*. The actual figure for the *ec-ct* distance is 18.4 per cent, which is very close, although not quite equal, to the sum of the *ec-cv* and *cv-ct* distances. This small discrepancy must be accounted for, but meanwhile the arrangement of the loci in the chromosome, based on the data discussed so far, may be taken to be as follows:

$$\begin{array}{ccccccc}
 & sc & & ec & & cv & & ct \\
 & \underbrace{\hspace{1.5cm}} & & \underbrace{\hspace{1.5cm}} & & \underbrace{\hspace{1.5cm}} & & \\
 & 7.6 & & 9.7 & & 8.4 & & \\
 & & & 10.2 & & & &
 \end{array}$$

The discrepancy just noted arises because the wild-type and *ec cv ct* flies in Table XXIII are formed when crossing over occurs both between the loci of *ec* and *cv* and between *cv* and *ct*; these flies arise through *double crossing over*. Since the occurrence of double crossing over restores the

parental combinations of the genes lying farthest apart, in our case *ec* and *ct*, the frequency of recombination of *ec* and *ct*, and hence the *apparent* distance between them, is smaller than the sum of the recombination frequencies between *ec-cv* and *cv-ct*. In general, the more remote in the

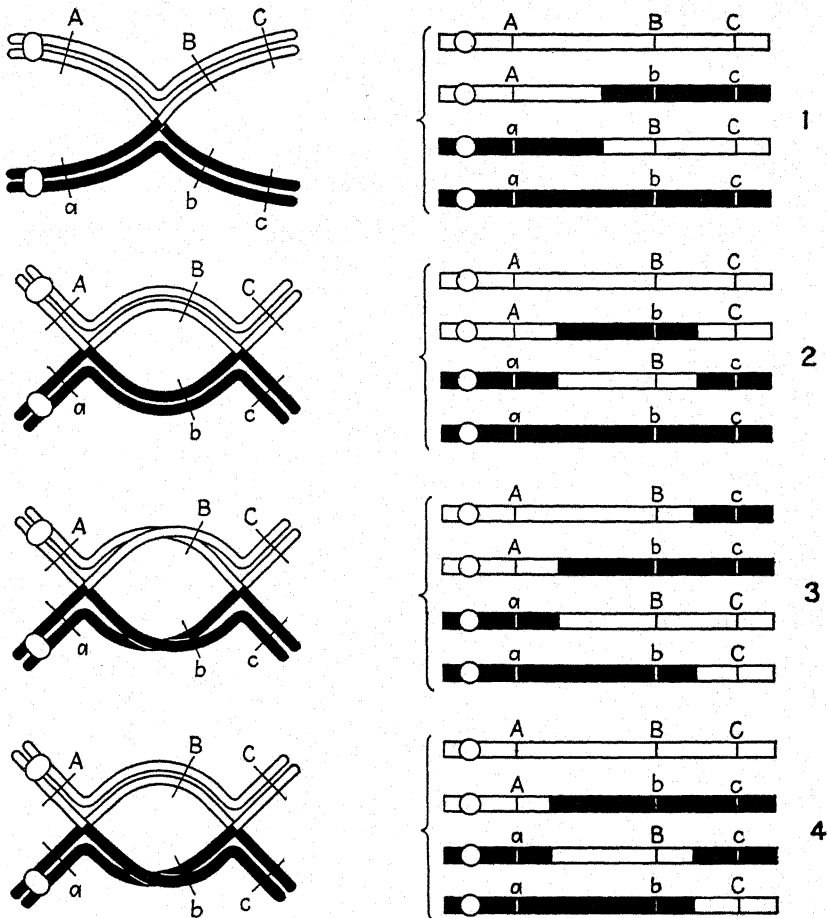


FIG. 94. Diagram showing chromosome bivalents with single chiasmata (above) and double chiasmata (below); 1, single chiasma; 2, two-strand double crossover; 3, four-strand double crossover; 4, three-strand double crossover.

chromosome the genes are, the more double crossing over takes place between them. With several linked genes involved in crosses, not only double but triple, quadruple, and even quintuple crossovers may be found. The multiple crossovers may cause wide departure from the rule that

frequencies of recombinations between remote genes should be equal to the sum of the recombination frequencies between the intervening genes.

We have seen above (p. 163) that crossing over is caused by exchange of sections of homologous chromatids at meiosis, visibly expressed in the appearance of chiasmata in the meiotic bivalents. A single crossover is, then, the product of a single exchange of sections between the chromatids; double or multiple crossing over results from a chromatid being involved in two or more exchanges (Fig. 94). The number of chiasmata per bivalent is, indeed, variable not only in different chromosomes and in different species but even in the same chromosome in different cells of the same individual.

Interference and Coincidence. In the experiment the results of which are summarized in Table XXIII, the frequency of single crossing over between the genes *ec* and *cv* is 10.1 per cent and between *cv* and *ct* 8.3 per cent. If the occurrence of crossing over in one part of a chromosome were independent of its occurrence in other parts of the same chromosome, we could predict very easily the frequency of simultaneous occurrence of crossing over between *ec* and *cv* and between *cv* and *ct*. On the assumption of independence, the frequency of such double crossing over should be 10.1 of 8.3 per cent, or .84 per cent. In actuality, the observed frequency of double crossovers is only .15 per cent (Table XXIII). In the experiment reported in Table XXII no double crossovers at all are observed (no wild-type and no *sc ec cv* flies). It seems, then, that the occurrence of crossing over at one point in the chromosome decreases the probability of its occurrence elsewhere in the same chromosome. This phenomenon is called *interference*. A measure of the degree of interference is called *coincidence* and is computed simply as a ratio of the observed number of double crossovers to the expected number of such doubles. For the data in Table XXIII, this ratio is evidently $.15/.84 = .18$, in other words, only 18 per cent of the expected doubles are actually found. For the data in Table XXII, coincidence is evidently zero, since no double crossovers at all are found.

In general, interference is greatest over short distances in the chromosomes, so that within a certain minimal distance there is no double crossing over (coincidence = 0). Farther apart, interference diminishes and at a certain distance disappears entirely (coincidence = 1). This might mean that at meiosis the chromosomes do not coil tightly about one another but are somewhat rigid, so that if a chiasma is established between the loci of the genes *scute* and *echinus*, no other chiasma can appear between *echinus* and *crossveinless* (Table XXII). What takes place at crossing over is exchange of *blocks of genes*, arranged in a linear order; these blocks may have certain characteristic lengths depending on the chromosome

and the particular region thereof. Interference is important in placing genes in the genetic chromosome maps, since where recombination data on distant genes must be used, the figures must be corrected for double crossing over before they can be taken to represent the "distances" between the genes.

Recombination and Crossing Over. The exchanges between homologous chromosomes (crossing over) provide the most reasonable physical mechanism for the recombinations of genes which occur between the members of a linkage group. This assumption served as an efficient and successful guide in planning and interpreting breeding experiments for many years, before direct demonstrations were given both in *Drosophila* (Stern) and maize (McClintock and Creighton) that a recombination is actually accompanied by crossing over or exchange of chromosome segments. Since the proof involved the use of certain chromosomal aberrations which are discussed in the next chapter, it is described in connection with the cytological chromosome maps (p. 263).

One important fact about crossing over that has been established by cytological observation and then confirmed by breeding experiments is that the exchanges of chromosome segments which cause recombination of linked genes occur at the four-strand, or tetrad, stage, in the prophase of meiosis. At this time, the homologous chromosomes have paired, each homologue has split into two chromatids which are held together by the undivided centromere, and chiasmata are observed in the tetrad (Fig. 94). A careful examination shows that at each chiasma only two of the four chromatids cross over, while the two other chromatids preserve their original continuities. In genetic terms, this must mean that of the four chromosomes which emerge from meiosis only two can show crossing over at any one level. In most animals and plants it is impossible to determine by observing the gene recombination in linkage experiments whether the crossing over which produces this recombination involved two, three, or all four strands of chromatids. The difficulty is that the products of each meiosis, the four haploid nuclei each with one chromatid of the original four, cannot usually be identified among the gametes, in the mass of which the cells coming from different meiotic divisions are inextricably mixed. But in some of the lower plants all of the cells derived from a single meiotic division remain together, and the individual gametes can be recognized, separated, and tested individually.

In the bread mold (*Neurospora*) each ascus, or fruiting body, contains 8 haploid ascospores as in Fig. 95, which have arisen from a single diploid cell through the two meiotic divisions, followed by one mitotic division. Dodge showed that these spores are disposed in a regular order in the ascus and developed a technique by which they can be dissected out and grown

separately, giving rise to haploid individuals which show the genetic constitution of each gamete. Lindegren, working with mutant genes in *Neurospora*, then showed that, when two pairs of linked genes undergo crossing over, exchange in any one region occurs only between two of the four chromatids, that is, between one chromatid of one homologue and one

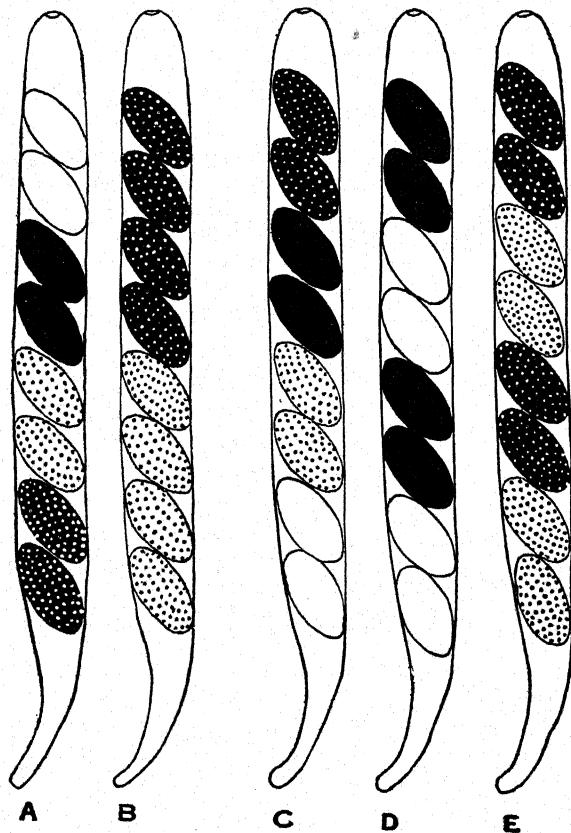


FIG. 95. Segregation in asci of *Neurospora sitophila*. Black and white spores indicate segregation for sex; dotted and plain, indicate presence or absence of conidia. Thus in C, segregation for sex occurred at the first division and for conidia formation at the second division. (From Dodge.)

chromatid of the other homologue, as in the diagram in Fig. 96. The proof that this is what happens is the fact that the four cells resulting from meiosis consist of two with parental combinations (noncrossovers) and two with recombinations of genes (crossovers) which could arise only if two of the four strands exchanged parts between the two loci being studied.

The same type of proof that crossing over between two loci involves only two of the four chromatids has been obtained from cases in *Drosophila*

in which some of the products of meiosis can be identified because of chromosome irregularities. In the case of nondisjunction of X chromosomes heterozygous for two mutant genes, it sometimes happens that a

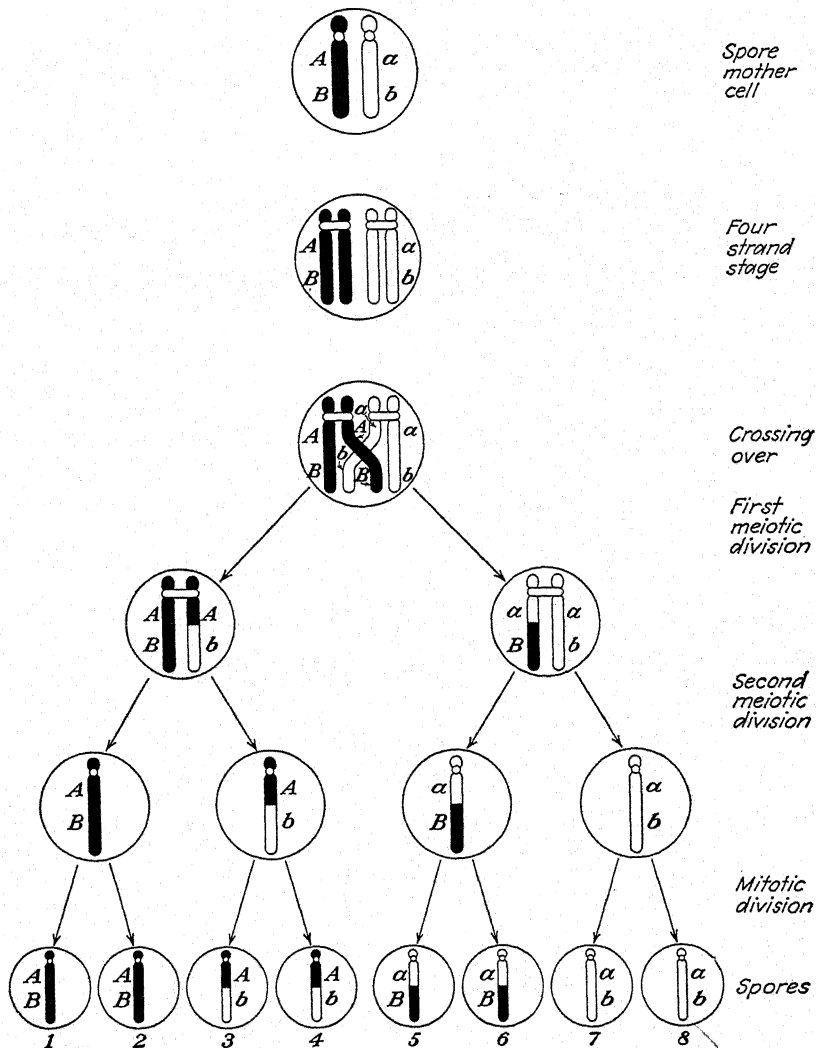


FIG. 96. Scheme of meiosis in *Neurospora*. Only one pair of chromosomes is shown.

crossover chromatid (such as *Ab*) and a noncrossover chromatid (such as *ab*) are found in the same nondisjunctional egg, which has thus two X chromosomes. If crossing over had occurred between whole chromosomes, no noncrossover strands would be found; hence this also indicates crossing

over between chromatids. Similar evidence has been obtained from trisomic chromosomes with linked genes in maize.

22p ✓ **Factors Affecting the Strength of Linkage.** The frequency of crossing over between genes and hence the frequency of chiasma formation between their loci in the chromosome may be influenced by a number of physiological and environmental factors. Thus, with increasing age of the female of *Drosophila melanogaster*, the amount of crossing over in her eggs becomes smaller. Furthermore, this effect is not equally pronounced in all chromosomes but is particularly noticeable in the middle portions of the second and third chromosomes in the neighborhood of the loci of the centromeres (see pp. 263-265). Temperature, X rays, and chemical composition of the food are other agencies that modify crossing over frequencies.

The frequency of crossing over in *Drosophila* males is so low as to be negligible under normal circumstances, but Whittinghill has shown that it can be increased by X rays. Although crossing over normally occurs only in gametogenesis or sporogenesis during meiosis, under some exceptional circumstances, which are not understood as yet, it may take place in somatic cells. This *somatic crossing over* has been studied by Stern in *Drosophila* and by Jones in maize. It results in the appearance of "twin spots" of tissue (Fig. 97) somewhere in the body which show complementary genetic constitutions. Suppose, for example, that a *Drosophila* female carries in one of her X chromosomes the gene for singed bristles, *sn*, and in the other X that for yellow body, *y*. Such a female has, of course, normal bristles and a gray body, because the genes *sn* and *y* are recessive to their normal alleles. But if a cell of this constitution, + *sn/y* +, undergoes a meiosislike process, cells homozygous for *y* and others homozygous for *sn* will be produced. A resulting fly will be in general normal, but it will have on some part of the body a yellow spot with normal bristles and next to it a spot with singed bristles but of normal color.

Measurement of Linkage from F_2 Data. The simplest technique of studying linkage is to cross strains differing in two or more linked genes, to obtain F_1 hybrids heterozygous for these genes (AB/ab or Ab/aB), then to cross these hybrids back to a strain homozygous for the recessive alleles of the genes tested (ab/ab). In the progeny of the test crosses, individuals with parental combinations and with recombinations of the genes are counted, and the frequency of recombination is expressed in percentages of the total number of individuals examined.

In some organisms, test crosses are much more difficult to obtain than F_2 progenies. For example, in wheat and some other grasses, crossing requires emasculation of many flowers, which upon artificial pollination produce only a few seeds each. But if the flowers are protected from foreign pollen, they are self-pollinated automatically and many F_2 seeds

are formed without further labor on the part of the experimenter. If the parents crossed differ in linked genes A and B and their alleles a and b , the F_2 generation will consist of individuals in the phenotypic classes AB , Ab , aB , and ab . Suppose that the numbers of individuals in these phenotypic classes are a , b , c , and d , respectively. Then from parents $AABB \times aabb$ (coupling) the parental (noncrossover) and recombination classes will be ad and bc , while in the opposite cross (repulsion $AAbb \times aaBB$) they will be ac and bd . The recombination fraction or linkage value, X , will be a function of the ratio $X = bc/ad$ for coupling, and $X = ac/bd$ for repulsion. Methods for calculating linkage values from these F_2 ratios have been



FIG. 97. A "twin spot" on the surface of a maize kernel, showing different colorations of the aleurone cells, due to somatic segregation. (After Jones.) (Courtesy of Connecticut Agricultural Experiment Station.)

worked out by Fisher and Balmukand (1928) and tables relating the ratio X above with linkage values have been supplied by Stevens (1939). For other methods concerning linkage calculations, the book of Mather (1938) should be consulted.

✓ **Linkage Maps.** The extensive study of linkage and crossing over, at first in *Drosophila* species and later in other organisms, led to the establishment of two important general principles. The first of these, the limitation of the linkage groups to the number of pairs of chromosomes characteristic of the species, is a corollary of the location of genes in chromosomes. The second, the theory of the linear order of genes, could hardly have been predicted from the above principle and required proof which was supplied by Sturtevant and later developed by Sturtevant, Muller, and

others to form the basis for a new method of representing the data of gene relationships in the chromosome obtained from studies of crossing over. This method of representation has been referred to as chromosome mapping and the maps constructed from crossover data as linkage maps, crossover maps, or genetical maps. Essentially they are condensed graphical expressions of the relative "distances," expressed in crossover percentages among the genes in one linkage group, as described on pages 216 to 220.

The actual construction of the linkage maps of *Drosophila melanogaster* represents the outcome of a collective effort of many investigators led and coordinated chiefly by C. B. Bridges from the inception of the work until his death in 1938. The maps have gradually included more and more gene loci, and the positions of these loci have become more and more accurate as more carefully controlled crossover data accumulated. One of the last maps constructed by Bridges is shown in Fig. 98. The genetic lengths of the four chromosomes, measured in terms of percentage frequencies of crossing over between genes, are 66 units for the X chromosomes, 107.3 for the second, 106.2 for the third, and only 0.2 unit for the fourth chromosomes. This corresponds fairly well to the lengths of these chromosomes as observed under a microscope, except that in the fourth chromosome crossing over seems to be excessively rare. It will be asked how distances in excess of 50 units can be possible, since crossing over may vary only from nearly zero (no crossing over) to nearly 50 per cent (independent assortment). It is true that the *amount of recombination* between two genes never exceeds 50 per cent, but, because of double crossing over, it is necessary to measure long distances by adding together the sums of the recombination values for intermediate genes, as explained above. These sums frequently exceed 50 in the long chromosomes. In the second chromosome, for instance, the genes for "Star" and "speck" appear on the map as 105 units apart. When Star and speck are crossed, they show less than 50 per cent of crossing over (actually about 48.7 per cent), but this is known to be due to the reduction caused by double crossing over. When the intermediate percentages are added, the sum is in excess of 100, which expresses the true distance between these two genes.

"Map distance," therefore, does not always correspond to crossover percentage as measured directly, and consequently the amount of crossing over between two genes cannot be read directly from the maps, except with genes so near together that no double crossing over occurs in the distance between them.

Linkage Maps of Maize Chromosomes. The most extensively mapped species, after *Drosophila melanogaster*, is the maize plant. Here, thanks to the cooperative work of many geneticists and plant breeders, under the leadership of R. A. Emerson, the locations of several hundred gene loci

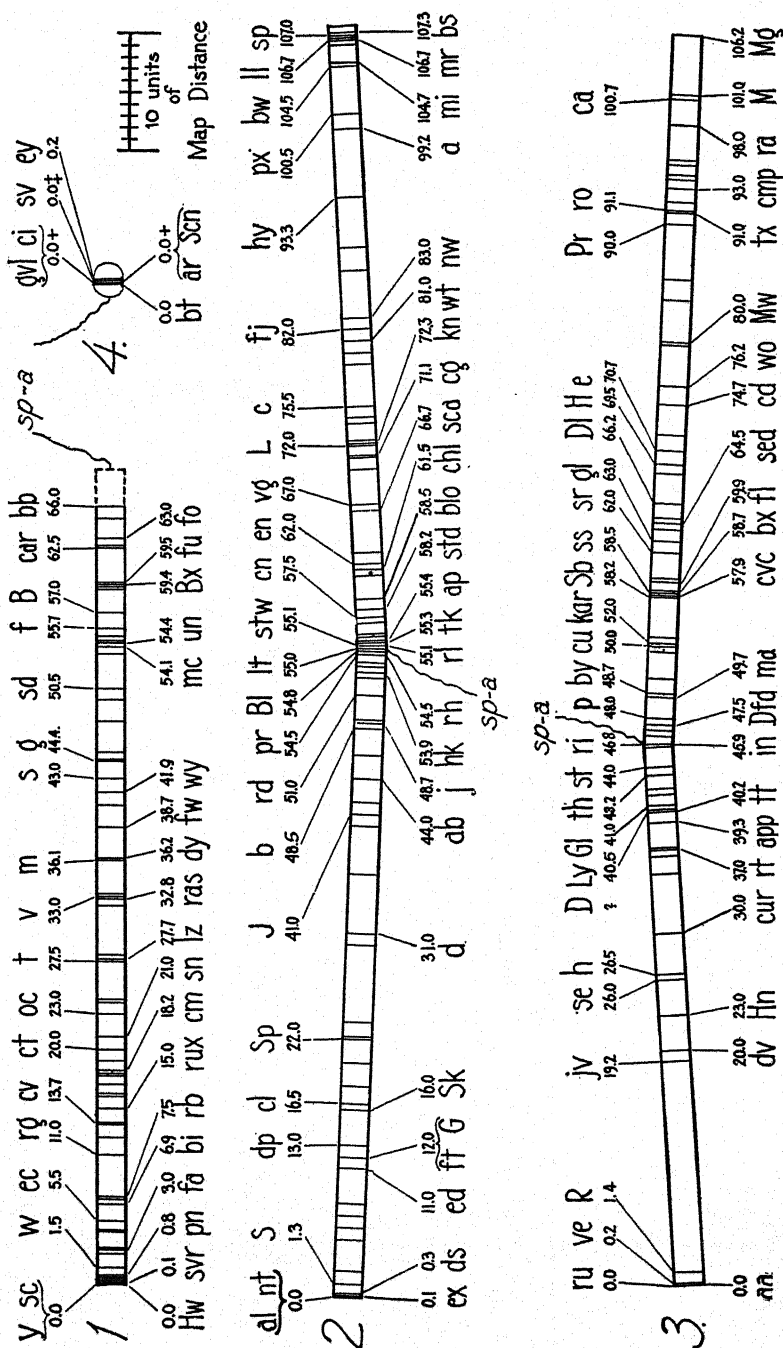


Fig. 98. Genetical map of the four chromosomes of *Drosophila* showing the positions of the more important gene loci. Figures refer to distances from the left end of the chromosome as determined from percentage of recombination as observed in linkage experiments. Symbols refer to loci listed and described in Table XXI. Loci above the chromosome are those most useful in genetic experiments. Spindle attachment points are indicated by *sp-a*. (After Bridges.)

Fig. 99. Linkage maps of the 10 chromosomes of maize (*Zea mays*), with most important "marker" loci. (From M. M. Rhoades.)

are known. Each of the 10 linkage groups of this plant (Fig. 99) have been placed in a particular one of the 10 microscopically visible chromosomes, by means of the study of chromosomal aberrations of the kinds discussed in Chapter X.

Maps of Human Chromosomes. In man a beginning has been made in constructing a linkage map of one chromosome, the X, but this necessarily derives from methods unlike those employed with animals and plants, which can be used experimentally. It is based on the fact, first noted in

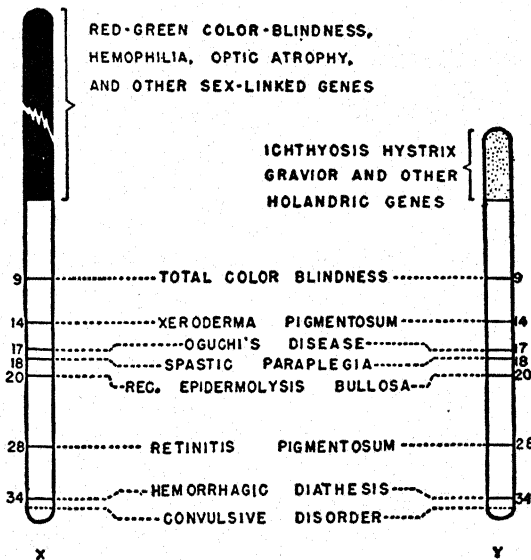


FIG. 100. Map of human X and Y chromosomes; the pairing segment shown in white, the differential segment of X chromosome shown in black, and the differential segment of the Y shown stippled. (From Snyder's "Principles of Heredity," by permission of D. C. Heath & Co., after Haldane.)

the aquarium fish *Lebistes* (the guppy), that occasionally a gene in the X chromosome (in *Lebistes* the male is the heterogametic sex, XY, undergoes crossing over and appears to be transmitted in the Y chromosome. Such genes have been called incompletely sex-linked. Thus the part of the X chromosome which contained this gene must have been homologous with a part at least of the Y. Later (1934) Koller and Darlington showed that chiasmata were probably formed between the X and Y chromosomes in spermatogenesis in man. It occurred to Haldane that, if the human X and Y chromosomes had homologous segments so that crossing over could occur between them, then genes in this segment which entered a zygote on the X chromosome would generally be passed on to offspring

in the X, but that, where crossing over had occurred, they would be transmitted in the Y and that the frequency of such exceptions would be a measure of the frequency of crossing over between the gene in question and the differential segment (Fig. 100) which carries the completely sex-linked genes. The pedigree in Problem 278 illustrates the method of detection of such incompletely sex-linked genes. The first male in the third generation got the gene from his mother, hence in the X, and passed it on to four daughters but not to three sons. On the assumption of incomplete sex-linkage, these would be the noncrossovers. However, one son got the gene from his father, hence in the Y, and two daughters failed to get it in the paternal X chromosome. These must have been crossovers. The analysis of many pedigrees led to an estimate of about 32.6 per cent of crossing over between this gene and the differential part of the X. A modification of the method permitted detection of additional incompletely sex-linked recessives and led to the construction of a map like that in Fig. 100. The accuracy of this map is not comparable with those for *Drosophila* species and maize, which are based on large numbers of experimentally controlled observations, but it is a useful approximation. Since the most reliable sex-linked mutant genes are rare, it is unlikely that two will often be found in the same pedigree; hence the construction of a map of the differential segment of the X is probably a matter for the distant future. One clear case of linkage of two autosomal genes, M-N blood type and sickle-cell anemia (Snyder, 1949) marks the beginning of the map of one of the 24 autosomes.

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PROBLEMS

240. Why should it be true that if crossing over is between chromatids, the percentage of chiasmata in a given region will be twice as great as the percentage of genetic crossovers there?

241. If two loci a and b are 10 map units apart, in what percentage of the gametes should a chiasma have been formed in this region?

242. If the average number of chiasmata per tetrad is two, what should be the maximum length of the genetic map of this chromosome?

243. Why should spindle-fiber attachment influence the amount of crossing over between chromatids?

244. How would you prove that purple flower color and dark stem color in *Datura*, which occur together, are due to a single gene rather than to two linked genes?

245. How would you determine whether characters which show no crossing over were due to alleles or to closely linked genes?

246. In *Drosophila* the genes for red, eosin, and white eye color are alleles. The gene for yellow body is linked with white eye with a crossover value of 1.5 per cent. What is the probable crossover value between eosin and yellow?

247. If a factor for high egg production and one for barring are both sex-linked traits, of what practical importance would this be to the poultry breeder?

Note. In problems involving linkage it is customary to designate the normal alleles of mutant genes by + (wild type). The genes in each member of a pair of chromosomes are written separately above and below a line (p. 216).

248. Assume that genes a and b are linked and show 40 per cent of crossing over. If a $\frac{++}{++}$ individual is crossed with one which is $\frac{ab}{ab}$, what will be the genotype of the F_1 ? What gametes will the F_1 produce, and in what proportions? If the F_1 is crossed with a double recessive, what will be the appearance and genotypes of the offspring?

249. If the original cross is $\frac{ab}{ab} \times \frac{a+}{a+}$, what will be the genotype of the F_1 ? What gametes will it produce? If the F_1 is crossed back with a double recessive, what will be the appearance of the offspring?

250. What will be the appearance of the F_2 ($F_1 \times F_1$) of the crosses described in the two preceding questions?

251. An individual homozygous for genes cd is crossed with wild type and the F_1 crossed back to the double recessive. The appearance of the offspring is as follows:

903 ++
898 cd
98 + d
102 c +

Explain this result, giving the strength of the linkage between c and d . If assortment between c and d were independent, what would be the result of this cross?

252. If the cross in the preceding question had been between a homozygous + d individual and a homozygous c + one, what would be the result of the cross of $F_1 \times$ the double recessive?

253. Calculate the percentage of crossing over between the factors for colorless aleurone and shrunken endosperm in corn from the combined data from both coupling (p. 205) and repulsion (p. 206) experiments.

Note. In *Drosophila* the mutant known as "black," b , has a black body in contrast to the wild type, which has a gray body; and the mutant "arc," a , has wings which are somewhat curved and bent downward, in contrast to the straight wings of the wild type.

254. From the data below calculate the crossover value between black and arc

I. Black, straight \times gray, arc

F_1 ♀♀ \times black, arc ♂♂ give:

Gray, straight.....	281
Gray, arc.....	335
Black, straight.....	335
Black, arc.....	239

II. Black, arc \times wild type

F_1 ♀♀ \times black, arc ♂♂ give:

Gray, straight.....	1,641
Gray, arc.....	1,251
Black, straight.....	1,180
Black, arc.....	1,532

Note. In *Drosophila* the mutant known as "vestigial," v , has wings which are very much reduced as compared with the long wings of the wild type.

255. In the two following crosses the parents are given, as in the previous question, together with the counts of offspring of F_1 females \times black, vestigial males (data from Bridges and Morgan):

I. Black, vestigial \times wild type (gray, long)

F_1 females \times black, vestigial males give:

Gray, long.....	822
Gray, vestigial.....	130
Black, long.....	161
Black, vestigial.....	652

II. Black, long \times gray, vestigialF₁ females \times black vestigial males give:

Gray, long.....	283
Gray, vestigial.....	1,294
Black, long.....	1,418
Black, vestigial.....	241

From these data calculate the crossover value between black and vestigial.

Note. In tomatoes Jones has found that tall vine is dominant over dwarf and spherical fruit shape over pear. Vine height and fruit shape are linked, with a crossover percentage of 20 per cent.

256. If a homozygous tall, pear-fruited tomato is crossed with a homozygous dwarf, spherical-fruited one, what will be the appearance of the F₁? of the F₁ crossed with a dwarf, pear? of the F₂?

257. What *genotypically different* types will there be in the F₂ of the preceding cross? What offspring will each of these produce if selfed?

258. A certain tall, spherical-fruited tomato plant crossed with a dwarf, pear-fruited one produces 81 tall, spherical; 79 dwarf, pear; 22 tall, pear; and 17 dwarf, spherical. Another tall, spherical plant crossed with a dwarf, pear produces 21 tall, pear; 18 dwarf, spherical; 5 tall, spherical; and 4 dwarf, pear. What are the genotypes of these two tall, spherical plants? If they were crossed, what would their offspring be?

Note. The inheritance of grain color in wheat is described on page 122.

259. What would be the F₂ ratio of red and white grains from a cross of red by white if two duplicate factors for red were linked, with a crossover value of 10 per cent.

Note. In rats dark eyes are due to the interaction of two genes *R* and *P*, the recessive allele of either producing light eyes. These genes are in the same chromosome.

260. When homozygous dark-eyed rats $\frac{++}{++}$ were crossed with double recessive ones $\frac{rp}{rp}$ and the F₁ crossed back with the double recessive, the following offspring were obtained (data from Castle):

Dark-eyed.....	1,255
Light-eyed.....	1,777

When $\frac{+p}{+p}$ animals were crossed with $\frac{r+}{r+}$ ones and the F₁ crossed back with the double recessive, the following offspring were obtained:

Dark-eyed.....	174
Light-eyed.....	1,540

Calculate the crossover value between *r* and *p*.

261. In *Drosophila* white eye color and club wing are both sex-linked with a

crossover value of about 15 per cent. If a wild-type female (red, long) is crossed with a white, club male, what will be the appearance of the offspring? If both males and females of the F_1 are crossed back to pure white, club stock, what will be the offspring in each case?

262. In the fowl assume that e (early feathering) and B (barring) are sex-linked and show 20 per cent of crossing over (in the male only). If a male from a cross of late-feathered, barred male \times early, black female is mated with an early, black female, what will be the appearance of their offspring, as to feathering and barring?

263. Assume that genes a and b are linked, with a crossover percentage of 20 per cent, and that c and d are also linked, with a crossover percentage of 10 per cent, but are in another chromosome. Cross a plant homozygous for $ABCD$ with one which is $ab cd$, and cross the F_1 back on $ab cd$. What will be the appearance of the offspring of this cross?

Note. In tomatoes red fruit color is dominant over yellow and is independent of the factors for height and fruit shape (for other data see Prob. 256).

264. Cross a homozygous tall, spherical-fruited, red-fruited plant with a dwarf, pear-fruited, yellow-fruited one, and then cross the F_1 back with a dwarf, pear, yellow. What will be the appearance of the offspring?

265. In sweet peas a cross of a homozygous, procumbent, hairy, white-flowered plant with a bush, glabrous, colored-flowered one produces an F_1 which is all procumbent, hairy, and colored-flowered. If this F_1 is crossed on a bush, glabrous, white-flowered plant, the offspring would be expected to show approximately the following distribution (data adapted from Punnett):

	Per cent
Procumbent, hairy, colored.....	6
Procumbent, hairy, white.....	19
Procumbent, glabrous, colored.....	6
Procumbent, glabrous, white.....	19
Bush, hairy, colored.....	19
Bush, hairy, white.....	6
Bush, glabrous, colored.....	19
Bush, glabrous, white.....	6

Explain these results, determining the strength of such linkages as may be observed.

266. In *Drosophila* yellow body is sex-linked and recessive to the gray body of the wild fly. Vermilion eye is also sex-linked and recessive to the wild red eye. The genes for yellow and vermillion show about 28 per cent of crossing over. The gene for vestigial wings is in one of the autosomes. If a homozygous yellow-bodied, red-eyed, long-winged female is crossed with a homozygous gray-bodied, vermillion-eyed, vestigial-winged male and if an F_1 female is crossed with a yellow, vermillion, vestigial male, what will be the appearance of the offspring of this last cross?

267. Assume that an individual homozygous for $++$ is crossed with one homozygous for ab and that the F_2 from this cross is as follows:

334 $++$, 37 $+b$, 38 $+a$, and 87 ab

How different is this result from that which you would expect if assortment between a and b were independent? What is the linkage between a and b ? Test your result by determining χ^2 .

268. In sweet peas a cross between a homozygous bright-flowered, tendrill-leaved plant and a dull-flowered, acacia-leaved (tendriless) plant produced an F_1 , which was all bright, tendril. The F_2 from this cross was as follows (data from Punnett):

424 bright, tendril
99 dull, tendril
102 bright, acacia
91 dull, acacia

The cross of bright, acacia on dull, tendril also gave an F_1 which was all bright, tendril, but the F_2 in this case was as follows:

847 bright, tendril
298 dull, tendril
300 bright, acacia
49 dull, acacia

What is the percentage of crossing over between these genes?

269. $AA\ BB \times aa\ bb$ gives the following segregation in F_2 :

AB	Ab	aB	ab
582	172	169	77

Do you think that a and b are linked or independent? Give evidence for your answer. Compare the actual distribution with the theoretical expectation on the basis of (1) independent assortment of a and b ; (2) 40 per cent crossing over between a and b , using the χ^2 test.

270. In *Nicotiana*, assume that the genes for colored or white flowers (C - c) are in the same chromosome with the self-incompatibility locus, s , and show 20 per cent crossing over with it. Compare the results to be expected from the following crosses:

- (a) $Cs^1/cs^2 \times Cs^3/cs^4$
(b) $Cs^1/cs^2 \times cs^2/Cs^3$

271. From the following data determine the order of the genes j , ms_8 , and v_{16} in maize (R , repulsion; C , coupling; B , backcross; S , selfed):

Genes		Linkage phase	Numbers of individuals				
X	Y		XY	Xy	xY	xy	Total
J	V_{16}	RB	82	565	542	71	1,260
J	V_{16}	RS	354	149	154	4	661
J	Ms_8	CS	464	39	23	135	661
Ms_8	V_{16}	RS	337	150	171	3	661

272. In *Drosophila*, white eyes (w), miniature wings (m), and forked bristles (f) are sex-linked and recessive to the wild-type characters red eyes, long wing, and straight bristles. In a cross of $\frac{wf m}{wf m} \times + + +$ the F_1 females crossed with $wf m$ males gave the following:

	Per cent
White, forked, miniature	26.8
Red, straight, long	26.8
White, straight, long	13.2
Red, forked, miniature	13.2
White, straight, miniature	6.7
Red, forked, long	6.7
White, forked, long	3.3
Red, straight, miniature	3.3

(a) Designate noncrossover, single-crossover, and double-crossover classes.

(b) Determine the percentage of crossing over between white and forked, white and miniature, and miniature and forked, and from this determine the order of these genes in the chromosome.

273. From the data in the preceding problem, compare the percentage of crossing over between the two most distant genes with the sum of the percentages of crossing over between the two end genes and the center gene. Explain this difference. Construct a chromosome map of these genes.

274. In maize, F_1 plants from the cross of colored, shrunken, starchy \times colorless, full, waxy were crossed with colorless, shrunken, waxy plants, and the following progeny observed (data from Hutchison):

Colored, shrunken, starchy	2,538
Colorless, full, waxy	2,708
Colored, full, waxy	116
Colorless, shrunken, starchy	113
Colored, shrunken, waxy	601
Colorless, full, starchy	626
Colored, full, starchy	4
Colorless, shrunken, waxy	2

Map the positions of c , s , and w , and determine the coincidence.

275. In *Drosophila* the mutant "morula," m , has a peculiar eye modification in which the facets are more irregular in size, shape, and color than are those of the normal eye. (For descriptions of mutants "black" and "arc," see Prob. 254.)

In the four following crosses the genes for *arc*, *black*, and *morula* entered the crosses in all four possible combinations, as stated. The counts in each case are the results of mating F_1 females with *arc*, *black*, *morula* males. Only the recessive alleles are named, the normal dominant alleles being assumed to be present unless the recessive is mentioned. Thus "black" flies are $b + +$, possessing the dominant alleles of *arc* and *morula*. The four crosses are as follows:

- I. Arc, black, morula \times wild type; F_1 female \times arc, black, morula male
 II. Arc, black \times morula; F_1 female \times arc, black, morula male
 III. Black, morula \times arc; F_1 female \times arc, black, morula male
 IV. Black \times arc, morula; F_1 female \times arc, black, morula male

The results of these four back crosses are given below (data from Bridges and Morgan):

	Cross I	Cross II	Cross III	Cross IV
Wild type.....	613	95	3	164
Black.....	445	40	13	187
Arc.....	38	713	113	21
Morula.....	82	851	107	7
Arc, black.....	55	884	96	8
Black, morula.....	29	666	120	15
Arc, morula.....	467	33	14	187
Arc, black, morula.....	514	79	2	133

Determine the crossover percentage between black and arc, arc and morula, and black and morula. Map the chromosome for these three points.

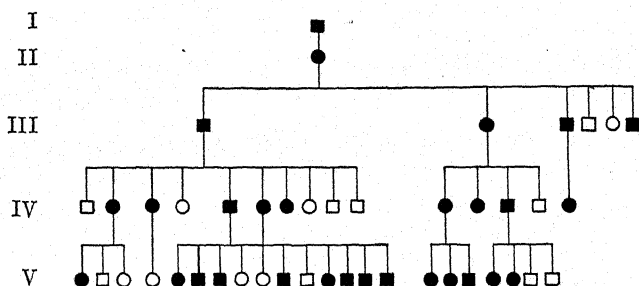
276. Below are the data from Bridges and Morgan for the crossovers between the genes black, curved, purple, speck, star, and vestigial in chromosome II of *Drosophila*. On the basis of the data, map the chromosome for these five genes as accurately as possible. Remember that determinations for short distances are more accurate than those for long ones.

Genes	Total flies	Crossovers
Black-curved.....	62,679	14,237
Black-purple.....	48,931	3,026
Black-speck.....	685	326
Black-star.....	16,507	6,250
Black-vestigial.....	20,153	3,578
Curved-purple.....	51,136	10,205
Curved-speck.....	10,042	3,037
Curved-star.....	19,870	9,123
Curved-vestigial.....	1,720	141
Purple-speck.....	11,985	5,474
Purple-star.....	8,155	3,561
Purple-vestigial.....	13,601	1,609
Speck-star.....	7,135	3,448
Speck-vestigial.....	2,054	738
Star-vestigial.....	450	195

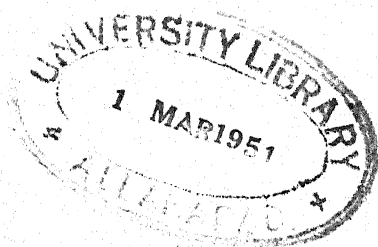
Locate also on this map the genes for arc and morula, studied in Problem 275. (Arc and morula are on the opposite side of black from star.)

277. In rats, two genes r and p (referred to in Prob. 260) are linked. $RR pp$ animals have pink eyes and light-colored coats; $rr PP$ animals have red eyes and light-colored coats. $RR PP$ animals have dark eyes and dark coats. Albinism, cc (pink eyes and white coat), is also linked with r and p . Design an experiment to measure this linkage and to map the chromosome containing r , p , and c , giving all necessary steps and crosses.

278. From the following pedigree of dominant retinitis pigmentosa, give the probable genotypes of all individuals, showing whether the mutant gene is probably in the X or Y chromosome and discriminating crossovers and noncrossovers.



Transmission of a dominant gene for retinitis pigmentosa through five generations. The mates of all persons shown were free from defect. (From Haldane after Snell.)



CHAPTER X

CHROMOSOME ABERRATIONS AND CYTOLOGICAL MAPS OF CHROMOSOMES

The linkage maps described in the previous chapter are essentially summaries of a large amount of statistical data on recombination of linked genes. The data are expressed in terms of distances between gene loci in a linear system. It is interesting to speculate that, even if chromosomes as bodies visible under a microscope were unknown, the data of the linkage maps might lead a biologist to infer that there must be, in the germ cells at least, bodies with the essential properties and behavior which chromosomes are known to possess. Indeed, the phenomena of segregation and independent assortment presuppose just such a mechanism as meiosis has proved to be. Similarly, the fact that the genes of each species form a specific number of linkage groups, each one a linear system, might lead one to suppose that the genes are arranged in single file in linear bodies.

It must now be obvious that microscopic observations on the behavior of chromosomes shed as much light on the transmission of genes as a knowledge of heredity does on cytological processes; and the actual history of genetics and cytology has been one of cooperation and mutual stimulation. As a result there have developed ingenious combinations of the techniques of experimental genetics and cytology. By these methods the evidence has been obtained which shows to what extent and in what ways the arrangement of genes in the linkage maps corresponds to their distribution in the actual chromosomes as revealed by the microscope. It is the chief purpose of this chapter to present and discuss this evidence.

Chromosome Morphology and Chromomeres. According to the theory of linear arrangement, a chromosome should be longitudinally differentiated into segments which correspond to individual genes. These gene segments, however, would be too small to be visible with any existing microscope, since the size of the gene is probably of the order of magnitude of a protein molecule (Fig. 101). Nevertheless, the ultimate differentiation of chromosomes into ultramicroscopic genes is often reflected on the cytologically visible level by the presence of constant structural features in the chromosome body.

It has already been pointed out (p. 159) that in many plant and animal species, particularly in those with large chromosomes, each chromosome of

the haploid complement is visibly different from every other one (Figs. 55 and 59, p. 158). The position of the centromere is often marked by a constriction in the chromosome, and this *primary constriction* is the place where the chromosome is frequently bent. The large autosomes (the sec-

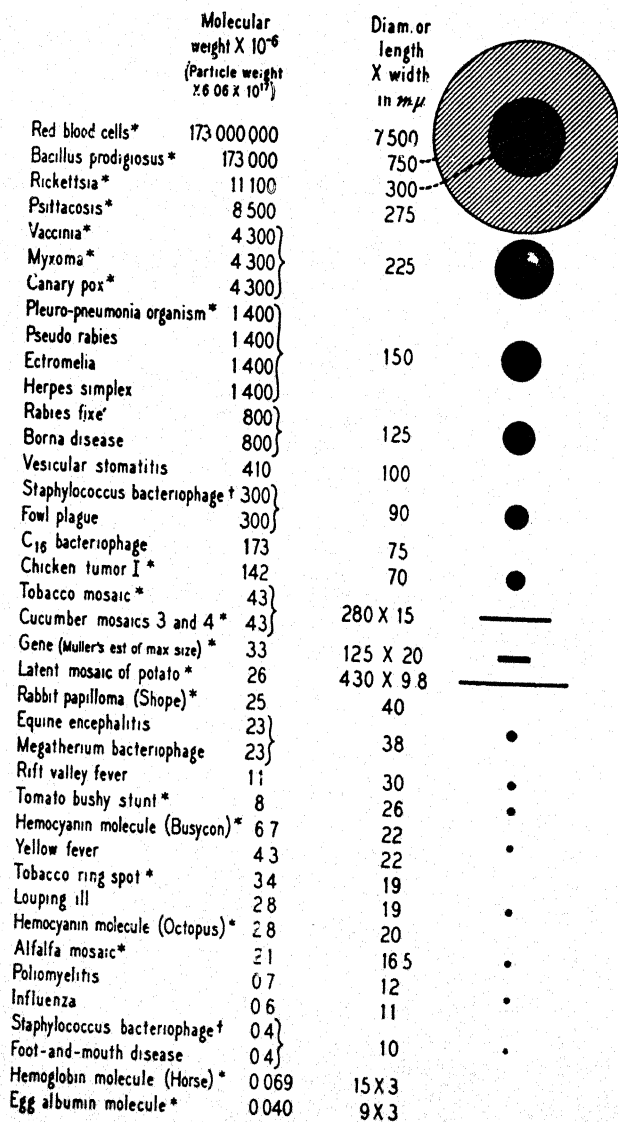


Fig. 101. Comparison of the size of different viruses, bacteria, and some large organic molecules for comparison with one estimate of gene size. (From Stanley.)

ond and third) of *Drosophila melanogaster* (Fig 69, p. 177) have centromeres located at about the middle of their length and therefore appear as V-shaped bodies at the metaphase and anaphase stages of mitosis. The Y chromosome is divided by the primary constriction into two unequal parts and therefore appears hook-shaped. The X chromosome has a subterminal centromere and is rodlike. A chromosome may have also one or more *secondary constrictions* at fixed places with respect to the free ends and the primary constriction. Chromosomes having several constrictions may appear to consist of several joints or segments.

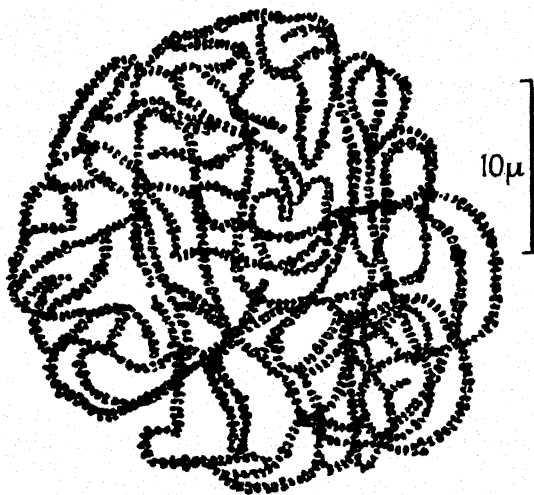


FIG. 102. Chromomeres in the meiotic prophase chromosomes of a lily. (From Belling.)

During prophases, and especially during the pachytene stage of the prophase of meiosis, the longitudinal differentiation in the body of a chromosome may stand out particularly clearly. A pachytene chromosome often resembles a chain of beads on a string. The beads, or *chromomeres*, are regarded by most cytologists as localized thickenings of the chromosome string, or *chromonema*. Some cytologists believe, however, that chromomeres are simply places where the chromonema is thrown into a tight, springlike coil, while the internodes connecting the chromomeres are places where the chromonema is relatively uncoiled and straightened. However that may be, the appearance of chromomeres attests the longitudinal differentiation of chromosomes in a very obvious manner. Belling (1928) studied with great care the pachytene chromosomes of a lily, *Lilium pardalinum* (Fig. 102), which show the chromomere structure in an especially clear way, and concluded that the number of the chromomeres in the haploid set is between 2,000 and 2,500. He was inclined to believe

that there might be a one-to-one correspondence between these "ultimate chromomeres" and genes, but this cannot be considered as proved. Another fact of importance discovered by Belling and other investigators is that adjacent chromomeres may differ from each other in size and that the seriation of chromomeres of different sizes is constant and characteristic for each chromosome. Furthermore, at the pachytene stage of meiosis, when homologous maternal and paternal chromosomes are paired, it may be seen that the chromomeres which are in contact are in fact of the same size and that the seriation of the chromomeres in the synapsed homologues

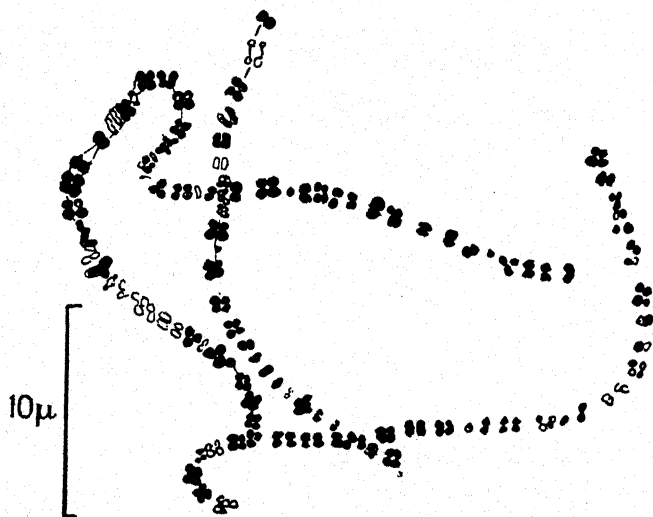


Fig. 103. Paired chromomeres in pachytene chromosomes of a lily. (From Belling.)

is, section by section, exactly the same (Fig. 103). This suggests very strongly that every chromomere is qualitatively distinct from every other one in the linear series and that the attraction forces which bring the chromosomes together and make them pair at meiosis are highly specific ones. The physicochemical nature of these specific attraction forces, which operate between homologous chromomeres in cells undergoing meiosis, is for the time being a matter of speculation.

Among organisms that are important as materials for genetic investigations, maize is remarkable in having chromosomes which show many structural details at the pachytene stage of meiosis (Figs. 58, p. 160, and 59, p. 161). Each of the 10 pairs of chromosomes can be recognized by its length, the position of the centromere, which divides the body of the chromosome into two arms of more or less unequal length, and the pattern, which is formed by the arrangement of larger and smaller, more and less

darkly staining chromomeres. The average lengths of the chromosomes and the ratios of the lengths of their longer and shorter arms are given in Table XXIV.

TABLE XXIV. AVERAGE LENGTHS, IN MICRONS, AND RATIOS OF THE TWO LIMBS (SEPARATED BY THE CENTROMERES), IN THE PACHYTENE CHROMOSOMES OF MAIZE AT MEIOSIS (After Longley from Rhoades)

Chromosome	Length	Length of the longer limb
		Length of the shorter limb
1	82	1.3
2	67	1.2
3	62	2.0
4	59	1.6
5	60	1.1
6	49	7.1
7	47	2.8
8	47	3.2
9	43	1.8
10	37	2.8

Giant Chromosomes in the Salivary Glands of Flies. Chromosomes of gigantic size, showing a wealth of structural details which permit not only every chromosome but even chromosome fragments to be recognized, occur in the salivary-gland cells of larvae of some species of Diptera. These remarkable chromosomes, which are important among the tools of modern genetic investigation, were discovered as early as 1881 by Balbiani in larvae of the midge *Chironomus*. But the significance of this discovery was not appreciated until 1933, when Painter, Heitz, and Bauer, and soon also Koltzoff and Bridges clarified the nature of these chromosomes and used them for studying genetical problems.

In *Drosophila*, as in other flies, the homologous members of each pair of chromosomes tend to lie side by side in the cell nuclei. This *somatic pairing*, which in many ways reminds one of the pairing which the chromosomes undergo in meiotic prophase, is so strong in the salivary-gland cells that the homologous chromosomes are tightly apposed and almost fused with each other. Furthermore, in the salivary-gland cells the chromosomes are 100 or more times longer than they are in other cells which are used to study *Drosophila* chromosomes, such as oögonia, spermatogonia, or neuroblasts in the larval brain. The salivary-gland chromosomes have the shape of long cylinders or ribbons, which consist of a succession of darkly staining disks or bands and of light internodes (Fig. 104). The darkly staining disks consist chiefly of a nucleoprotein containing desoxy-ribose nucleic acid; some of them are thicker or more darkly staining than

others; some consist of dots, while others appear solid. The pattern of disks is diagnostic for each section of each chromosome, as shown in the "maps" (Fig. 105). The centromere regions of all the chromosomes are associated together in a mass in which the bands appear to be less discrete than in other parts; this mass is called the *chromocenter*. Owing to the tight somatic pairing and to the association of the centromeres in the chromocenter, the correspondence between the chromosomes in the metaphase plates and in the salivary-gland cells is as shown in Fig. 106.

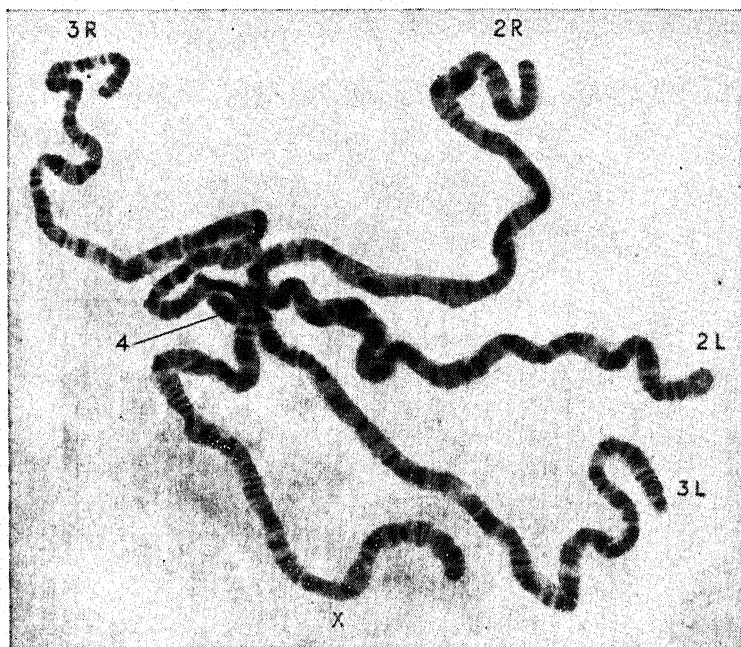


FIG. 104. Photomicrograph of nucleus of salivary gland cell of *Drosophila melanogaster* female showing the X, the right and left arms of large autosomes (2R, 2L, 3R, 3L), and the small fourth chromosome. (Courtesy of B. P. Kaufmann.)

Until 1939, at least 5,149 distinct disks had been counted in the salivary-gland chromosomes of *Drosophila melanogaster*, and it is likely that some faint disks had been overlooked (Bridges). A tempting, but unproved, working hypothesis is that there exists a one-to-one correspondence between the genes and the ultimate disks in the salivary-gland chromosomes.

The cells of salivary glands of fly larvae never divide, and they disintegrate soon after the larva pupates. The peculiar giant chromosomes observed in these cells appear to correspond to those seen at the prophase stage in ordinary cells and thus to constitute a "permanent prophase." The giant size of the chromosomes is believed by most cytologists to be due

to the chromonema, which in ordinary chromosomes is spirally coiled but in the salivary chromosomes is completely unwound, while the internodes between the chromomeres are lengthened. Moreover, the chromonema in the salivary-gland cells has undergone repeated division, without, however,

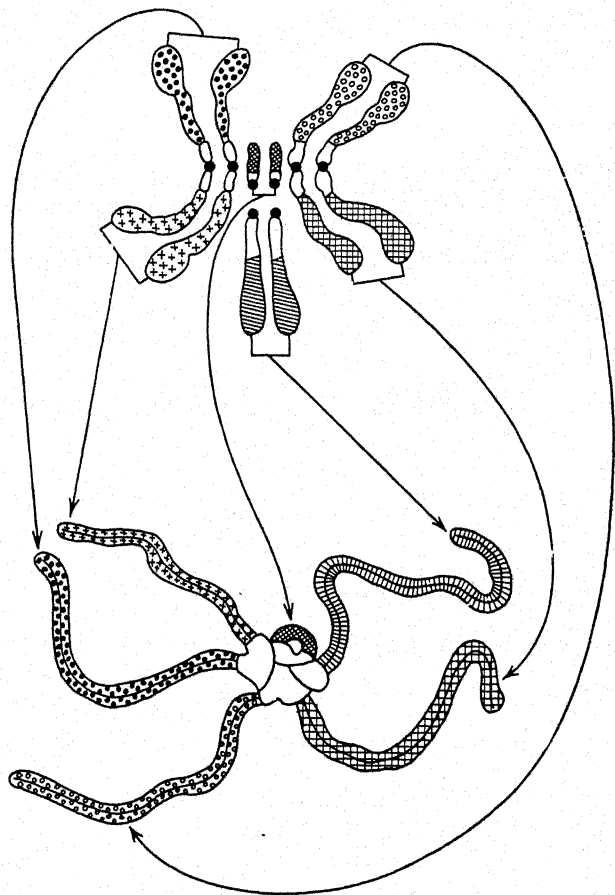


FIG. 106. A schematic representation of the chromosomes of *Drosophila melanogaster*, as seen at metaphase stage of mitosis in dividing cells (above) and in the cells of the salivary glands of fully grown larvae (below). Each chromosome "limb" is shown with different shading. The heterochromatic parts of the metaphase chromosomes, which form the "chromocenter" in the nuclei of the salivary-gland cells, are shown in white. The centromeres of the metaphase chromosomes (invisible in the salivary-gland cells) are shown in black.

either the nucleus or the cell having divided at a similar rate. Such a chromosome division, which is not accompanied by nucleus and cell division, is called *endomitosis* and is known to occur in certain tissues in both animals and in plants. Perhaps owing to the somatic pairing of chromosomes in flies, endomitosis in the salivary-gland chromosomes results in formation of

cabellike bundles of chromonemata, with the homologous chromomeres tightly paired to form the stainable disks, and the internodes forming the light segments between the disks.

Chromosomal Aberrations. The above cytological evidence strongly suggests that a chromosome consists of a succession of qualitatively distinct segments or links arranged in a linear sequence. Positive and conclusive evidence that this linear differentiation visible under a microscope actually corresponds to the linear arrangement of genes inferred from genetic experiments (see Chap. IX) has been provided by combined cytological and genetic studies of changes in the chromosome structure which arise from time to time in all organisms, both spontaneously (that is, without any known cause) and particularly under the influence of X rays (see Chap. XI). Changes in chromosome number or structure are called *chromosome aberrations*. They may be grouped under two main types, those which involve the number of the chromosomes and those which alter the number or arrangement of genes in the chromosomes.

I. Changes in the number of chromosomes.

A. Changes involving entire sets; n = basic, or monoploid, number.

1. *Haploidy* (n); each chromosome represented singly.
2. *Polyploidy*; each chromosome represented by more than two homologues. Triploidy ($3n$); tetraploidy ($4n$); pentaploidy ($5n$), etc. An autopolyploid is one derived by chromosome multiplication from a single diploid, so that the homologues come from the same source as in pure strains or homozygotes. An allopolyploid is one derived from a hybrid between two diploids, so that the homologues come from different sources.

B. Changes involving the numbers of chromosomes in a set (heteroploidy).

1. *Monosomics* represent the loss of one chromosome from one set. Where this occurs in the diploid, the chromosome complement is $2n - 1$.
2. *Polysomics* represent the addition of one or more chromosomes to one set. Trisomic = $2n + 1$, tetrasomic = $2n + 2$, etc. (More than one chromosome set may be affected; double trisomics = $2n + 1 + 1$, etc.)

II. Changes in the number or arrangement of gene loci within a chromosome (Fig. 107).

A. In number.

1. *Deficiency* or deletion—loss of one or more genes.
2. *Duplication*—addition of one or more genes, as a result of which the organism carries the same gene repeatedly in its haploid-chromosome complement.

B. In arrangement.

1. *Translocation*, or segmental interchange—exchange of parts between nonhomologous chromosomes to form two new chromosomes. For example, if the original chromosomes were ABCDEF and GHIJKL, the new ones may be ABCJKL and GHIDEF.
2. *Inversion*. Within a chromosome, a block of genes may rotate by 180 degrees. For example, a chromosome with genes in the order ABCDEFG may change to AEDCBFG.

The phenomena of heteroploidy have been discussed in Chapter VIII; polyploidy will be dealt with in Chapters XI and XIII. The other aberrations are to be considered in the following paragraphs.

Deficiency. A female with a notched wing margin was found by Bridges (1917) in a culture of *Drosophila melanogaster*. This trait, called Notch, was inherited as a sex-linked dominant which was lethal in the male, that is, male zygotes carrying Notch die. An unusual situation arose, however,

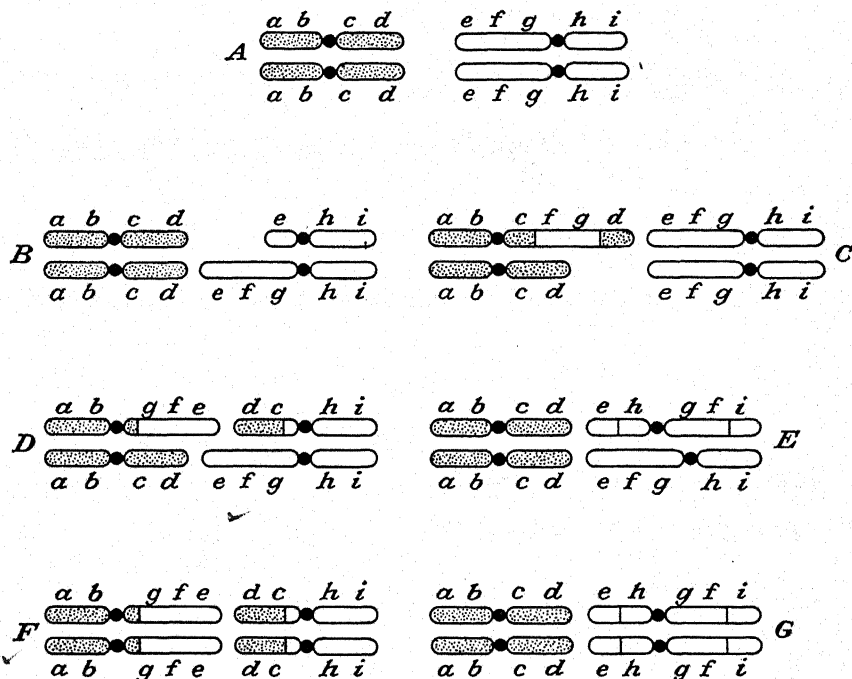


FIG. 107. Classification of chromosomal aberrations. A, normal chromosomes; B, deficiency; C, duplication; D, heterozygous translocation; E, heterozygous inversion; F, homozygous translocation; G, homozygous inversion. The centromeres are shown as black circles.

when Notch females with normal red eyes were crossed to white-eyed males (*w*, sex-linked recessive, see Fig. 71, p. 181), for the F_1 females with Notch wings had also *white eyes* (Fig. 108), as though the gene *white* were dominant in the presence of Notch. Mohr (1923) showed that some other sex-linked recessive genes lying in the vicinity of *white* on the linkage map (Fig. 98, p. 227)—for example, *facet*—also show this *pseudodominance* in the presence of Notch. A hypothesis was formulated according to which Notch arose as a result of a *loss of a piece of the chromosome* containing the loci of the genes which show the pseudodominance. This hypothesis was

confirmed when Mohr found that the frequency of crossing over between the genes lying to the left and to the right of the deficient piece was decreased in Notch flies by 3.8 per cent, just as was to be expected if the

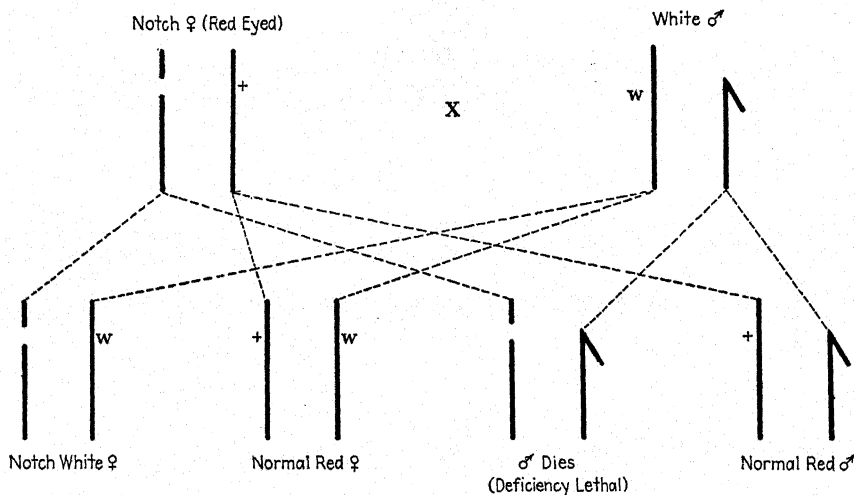


FIG. 108. The inheritance of Notch deficiency in *Drosophila melanogaster*.

deficient piece were so long that about 3.8 per cent of recombination was due to crossing over in that piece.

Mohr was unable to confirm his hypothesis cytologically, because the section of the chromosome deficient in Notch is too small to produce an appreciable shortening of the X chromosome as seen at mitosis. Such a confirmation, for Notch and for other deficiencies discovered meanwhile, became possible when the technique of studying chromosomes in salivary-gland cells was introduced. In individuals heterozygous for a deficiency, one of the two paired homologous chromosomes is shorter than the other, and since the pairing occurs only between homologous disks, the section of the normal chromosome which contains the disks missing in the other chromosome forms a buckle, as shown in Fig. 109. Now, the genes which, on the basis of the genetic tests, are known to be missing in the deficiency must be located in the part of the chromosome which forms the buckle in the deficiency heterozygotes.

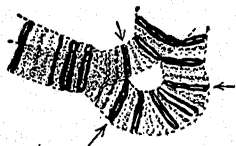


FIG. 109. Portions of salivary Chromosome II of *Drosophila* heterozygous for Notopleural, a deficiency for a series of bands (44E-45E). It is evident that in the deficient strand (above), the bands between the arrows on the normal one (below) are missing. (From Bridges.)

Thus, the position of certain gene loci is determined not in terms of a linkage map but in terms of the chromosome as seen under the microscope. By using this method, a number of genes have been

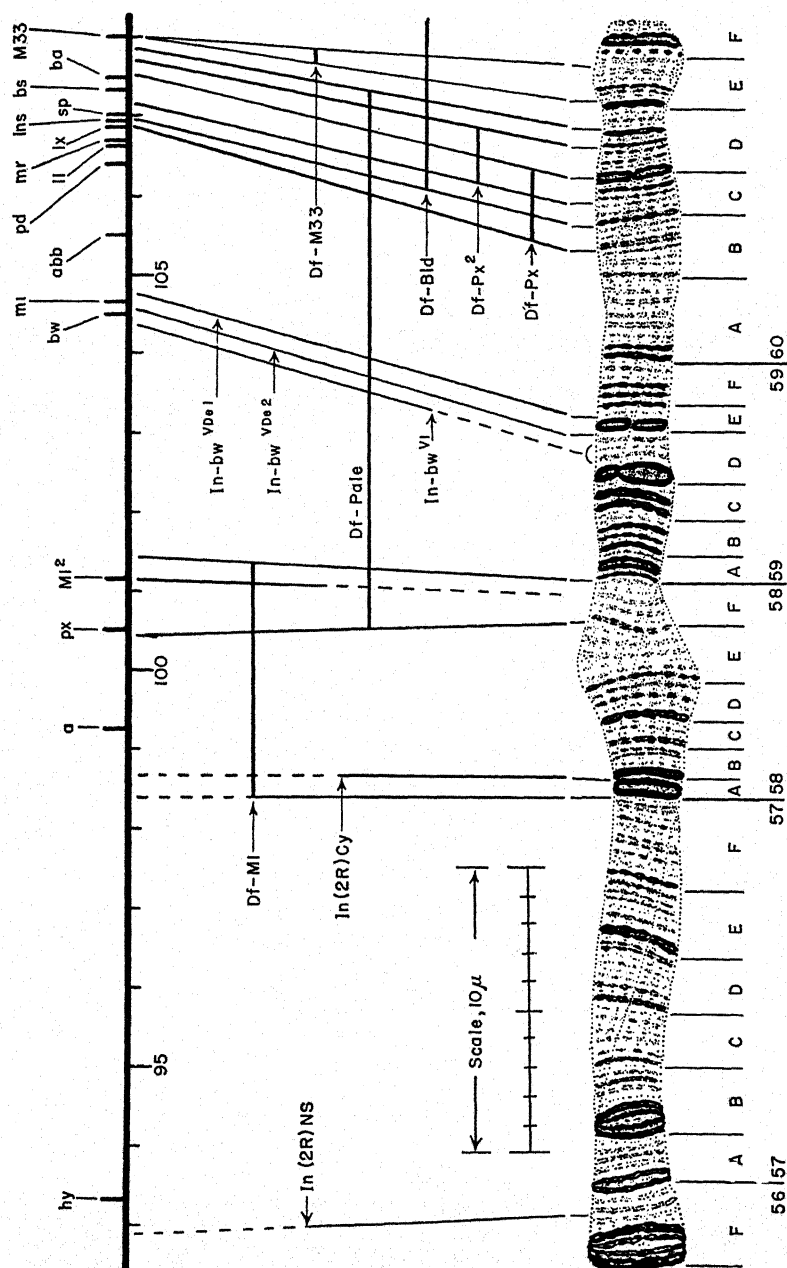


FIG. 110. A comparison of the genetic linkage map (above) and a corresponding section of the salivary-gland chromosome (below). This is the end portion of the right limb of the second chromosome of *Drosophila melanogaster*, cf. Figs. 98 and 105. (From Bridges.)

localized in the salivary-gland chromosomes of *Drosophila melanogaster* (Fig. 110). It is interesting that for some genes the localization has been narrowed to a very small group of disks, or even to a single disk, in a chromosome. Deficiencies have been seen also in the pachytene chromosomes in maize.

In *Drosophila*, only individuals heterozygous for short deficiencies, generally not exceeding a few dozen disks in the salivary-gland chromosomes, are viable. Even these individuals usually show some external abnormalities, such as the notching of the wing margin in Notch deficiency, which appear to be due to certain genes being present in a single dose, instead of in the normal double dose. On the other hand, deficiency homozygotes (that is, individuals in which certain genes are wholly missing) are, with very few exceptions, lethal. Demerec showed that homozygous deficiencies are usually lethal even if they arise in small groups of cells surrounded by normal tissues in which all the genes are present. This suggests that most genes are indispensable, at least in single dose, for the development of a viable organism.

Some species, however, do not show so extreme a sensitivity to deficiencies. In tobacco a complete collection of monosomics, that is, of individuals heterozygous for deficiencies of whole chromosomes, and in wheat a collection of nullosomics, individuals homozygous for deficiencies of whole chromosomes, have been obtained (see p. 197). These plants are, however, known to be polyploid, that is, they have the entire chromosome complement represented four times (in tobacco) or six times (in wheat) in somatic cells. Monosomics and nullosomics in polyploids are, hence not quite comparable to deficiencies in diploid organisms. In maize most deficiencies are unable to pass through the gametophyte, especially through the pollen grains, because the pollen grains in which a chromosome or a section is missing are inviable. But a few very short deficiencies in maize survive, even in homozygous condition, and produce phenotypically abnormal plants. Whether this means that some genes in maize are less indispensable for the organism than other genes or that maize has some genes represented several times in the chromosomal complement is not known.

Duplication. Bridges (1919) observed that some individuals of *Drosophila melanogaster*, which should have been homozygous for certain recessive genes, failed to manifest the effects of these genes in the phenotype. An analysis showed that dominant alleles of the genes in question were present in addition to, and at a different point in the chromosome from, the recessive ones. The flies evidently contained an extra piece of a chromosome, in addition to a complete diploid chromosome complement. Since, as a rule, one dominant allele of a gene is sufficient to suppress the effects of two recessives, the exceptional individuals with an extra piece of

chromosome showed the effects of the dominant alleles in the duplication. Cytologically, a duplication may produce the same kind of configuration in a chromosome as a deficiency does (Fig. 109, p. 248). Thus, in salivary-gland cells, one chromosome homologue is longer than the other, and it forms a buckle in pairing.

If a duplicating fragment of a chromosome includes the centromere, it may be present as a small extra chromosome, added to a normal chromosome complement (Fig. 111). Which genes are and which are not repre-

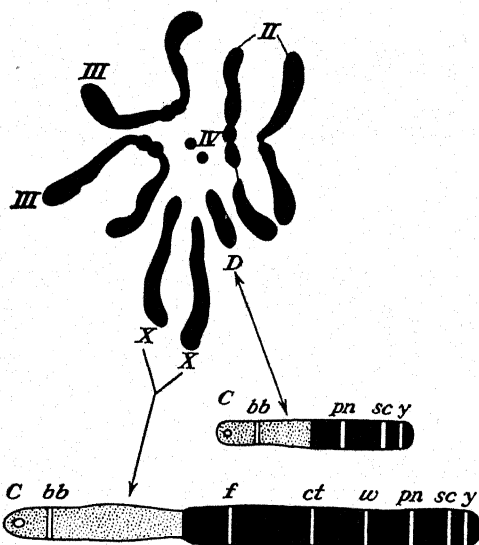


FIG. 111. A duplication for a section of the X chromosome of *Drosophila melanogaster*. The duplication (*D*) contains its own centromere, and therefore appears as a separate extra chromosome. X, II, III, and IV are the X, second, third, and fourth chromosomes, respectively. Below is a schematic representation of the duplication and of a normal X chromosome; the heterochromatic parts are shown stippled, and the euchromatic parts are black; *bb*, *f*, *ct*, *w*, *pn*, *sc*, and *y* are the locations of some genes in the X chromosome; *C* is the centromere.

sented in the fragment can be determined genetically by observing the suppression or nonsuppression of recessive genes present in double dose in the normal chromosomes. Observations and measurements of the fragments under a microscope show, then, the size of the chromosome sections containing known gene loci.

The effects of duplications on viability are generally less deleterious than those of deficiencies, so that relatively long duplications survive. Individuals carrying duplications show, nevertheless, various abnormalities in bodily characters, and when these abnormalities are known, they may be used to identify the carriers of the corresponding duplications.

A careful study of the salivary-gland chromosomes in *Drosophila melano-*

gaster led Bridges (1935) to conclude that some blocks of genes are present more than once in the haploid chromosome complement. Such repeated sections, or *repeats*, have presumably arisen in the evolution of the chromosome complement of *Drosophila* by means of a process of duplication. The occurrence of repeats results in certain genes being present two or more times in the chromosome set. This creates a situation resembling that which obtains in polyploids; it is possible that the exceptional cases of viable deficiencies (see p. 250) are connected with the existence of repeats.

Translocation. Deficiencies and duplications involve losses or additions of genes to the normal gene complement, and hence the carriers of these chromosomal aberrations are as a rule distinguishable from normal representatives of the species to which they belong by their appearance. Translocations and inversions change only the arrangement of the genes in the chromosomes, but not the quality or quantity of the genes. For this reason they are sometimes referred to as *chromosomal rearrangements*. Individuals carrying such rearrangements should be phenotypically entirely normal unless the relations of a gene or genes to adjacent genes affect the phenotypic expression (*cf.* "position effect," p. 450). The first translocation was discovered by Bridges (1923) in *Drosophila melanogaster*.

The genetic techniques for detecting and studying translocations will be more easily understood when the cytological phenomena produced by translocations are known. Suppose that two chromosomes, having respectively the genes *ABCDEF* and *GHIJKL*, exchange sections and give rise to translocation chromosomes *ABCJKL* and *GHIDEF*. An individual is thus formed which receives from one of its parents the normal and from the other parent the translocation chromosomes. Such an individual is a *translocation heterozygote*. Since the chromosome pairing at the meiotic prophase, or in the salivary-gland cells, is caused by specific attraction of homologous sections containing allelic genes, a translocation heterozygote may be expected to produce a cross-shaped pairing configuration, represented schematically in Fig. 112. Such configurations have actually been observed in pachytene chromosomes of translocation heterozygotes in maize (Fig. 113) and in salivary chromosomes of *Drosophila* translocation heterozygotes (Fig. 114). It is a rather simple matter to identify cytologically the points in the chromosomes at which these chromosomes had broken to produce the translocation.

In organisms in which neither salivary-gland chromosomes nor pachytene chromosomes are favorable for study, translocations can be detected by means of observation of the chromosome configurations at the first meiotic division. Consider again the cross-shaped arrangement of the chromosomes formed at the pachytene stage of meiosis in translocation heterozygotes (Figs. 112 and 113, opposite). Occurrence of crossing over in

each of the four arms of the cross will result in formation of chiasmata in each arm. Instead of two bivalents, that is, of pairs of synapsed homologous chromosomes, there will be formed a *quadrivalent*, or a group of four associated chromosomes, each member of the group being partially homologous to two other chromosomes in the group. The quadrivalent will appear at diakinesis and at metaphase of the first meiotic division as a ring, or circle, of four chromosomes, which may be either twisted as shown in Fig. 115, left, or open as in Fig. 115, center. If chiasmata fail to be formed in one arm of the pachytene cross, the ring is transformed into an open chain of four chromosomes. Such rings or chains of chromosomes were observed

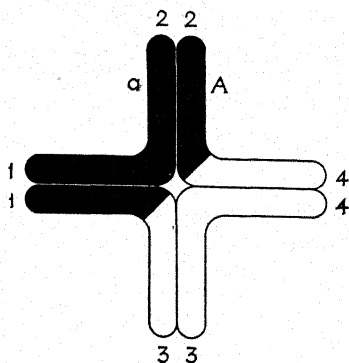


FIG. 112

FIG. 112. A diagram of chromosome pairing at meiosis in a translocation heterozygote.

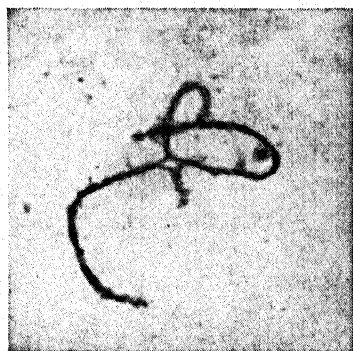


FIG. 113

FIG. 113. A photomicrograph of chromosome pairing at meiosis in a reciprocal translocation between Chromosome VIII and Chromosome X in maize. (Courtesy of M. M. Rhoades.)

and correctly interpreted by Belling at meiosis in the Jimson weed (*Datura*), and were thereafter found in maize, peas, wheat, spiderwort (*Tradescantia*), and other plants and in some animals, such as certain grasshoppers. They occur regularly in many evening primroses (see Chap. XI).

There are several ways in which the chromosomes associated in a ring or a chain may become distributed to the gametes formed as a result of meiosis (Fig. 115). The two original chromosomes, *ABCDEF* and *GHIJKL*, may go to the same gamete, and the translocation chromosomes, *ABCJKL* and *GHIDEF*, to another gamete. It may be noted that each of these gametes has every gene (symbolized by a letter) present once and only once, as gametes formed by normal individuals, that is, those not containing a translocation, usually do. On the other hand, if chromosomes adjacent in the ring go to the same pole at the meiotic division, the following four kinds of gametes are formed: *ABCDEF* and *ABCJKL*; *ABCDEF*

and *GHIDEF*; *GHIJKL* and *GHIDEF*; *ABCJKL* and *GHIJKL*. The common property of these four kinds of gametes is that they carry certain genes twice and do not have some genes at all. In other words, they carry duplications for some and deficiencies for other genes.

In most plants, pollen grains which contain deficiencies or duplications are as a rule inviable and are aborted (p. 250). Such pollen grains appear

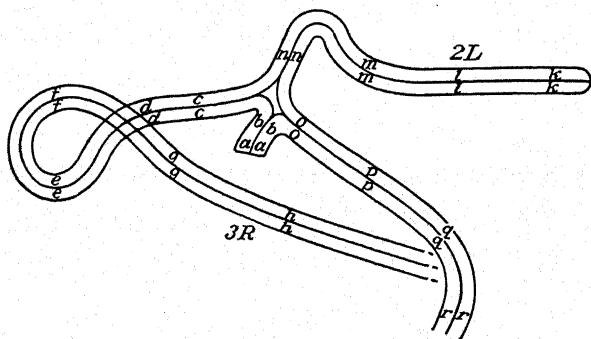


FIG. 114. Pairing between two normal chromosomes (2L and 3R) and two with reciprocal translocations in the salivary-gland nuclei of *Drosophila melanogaster*. In the diagram 2L has genes *klmnopqr*, 3R has genes *abcdefgh*, 2L3R has genes *abopqr*, 3R2L has genes *klmncdefgh*. (After Demerec and Kaufmann.)

empty and shriveled and can be clearly distinguished from pollen grains which have normal chromosomal complements. Embryo sacs with deficiencies and duplications may also abort. Consequently, plants heterozygous for translocations may produce fewer seeds than normal, and such plants appear *semisterile*. The semisterility of translocation heterozygotes furnishes one of the simplest and most practical ways of detecting translocations in maize. It must be kept in mind, however, that semisterility may be produced by lethal genes as well as by translocations and that in some plants, for example some evening primroses, translocation heterozy-

gotes may give rise almost entirely to gametes with normal gene complements.

There is a very interesting difference between the behavior of translocation heterozygotes in plants and in animals. In maize, pollen grains

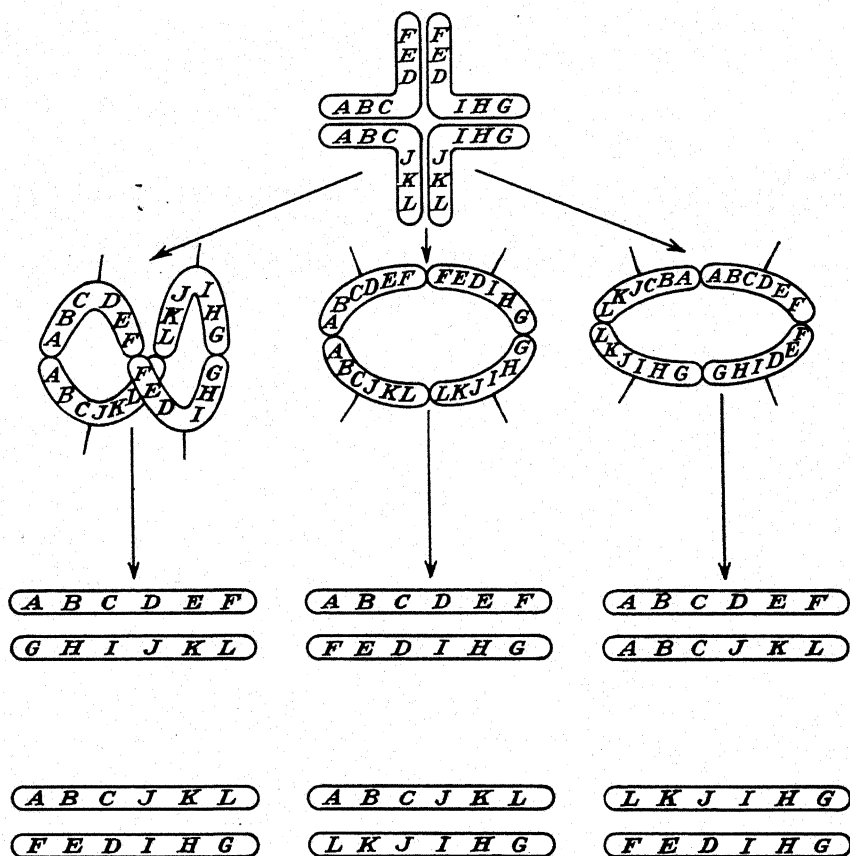


FIG. 115. Reduction division in a translocation heterozygote and gametes formed as a result of it. Topmost figure, the cross-shaped configuration formed at the pachytene stage of meiosis in a translocation heterozygote. Second row from the top, the twisted ring and the two open ring configurations formed by the chromosomes involved in a translocation heterozygote at meiotic metaphase. The two lower rows, the chromosomal complements in the six types of gametes formed by a translocation heterozygote; note that only the two types of gametes represented on the left contain normal sets of genes, while each of the other four contain some genes in duplicate (duplication) and do not carry some genes at all (deficiency).

and megaspores with deficiencies and duplications for blocks of genes abort; in *Drosophila*, eggs and spermatozoa with such abnormalities function normally and form zygotes (fertilized eggs) which lack certain genes and carry certain genes in excess. These abnormal zygotes die, so that

translocation heterozygotes in animals are effectively semisterile, just as they are in plants. But the ability of spermatozoa to function despite gross abnormalities in gene contents has led Muller and Settles (1927) and other investigators to conclude that *genes do not function* in the spermatozoa, at least in some animals.

Suppose now that a *Drosophila* male heterozygous for a translocation is made also heterozygous for some genes in the chromosomes involved, thus (the black line symbolizing a chromosome):

$$\begin{array}{cccccc} A & B & C & D & E & F \\ \hline A' & B' & C' & J' & K' & L' \end{array} \qquad \begin{array}{cccccc} G & H & I & J & K & L \\ \hline G' & H' & I' & D' & E' & F' \end{array}$$

Since there is no crossing over in the *Drosophila* male, the only two kinds of gametes that will be free from deficiencies and duplications will be (1) *ABCDEF* and *GHIJKL*, and (2) *A' B' C' J' K' L'* and *G' H' I' D' E' F'*. Gametes with all other combinations of chromosomes will give rise to inviable eggs, as shown above. This amounts to saying that all the genes symbolized by the letters without primes and all the genes symbolized by the letters with primes will be *completely linked*, despite the fact that they are carried in two different chromosomes. This apparent linkage of genes known to belong normally to different linkage groups offers a method for detecting translocations in genetic experiments, without the aid of a microscope. For example, in a certain experiment, 121 *Drosophila melanogaster* males heterozygous for the dominant genes Dichaete (*D*, certain bristles absent, wings spread) and Bristle (*Bl*, bristles short and stubby) were test-crossed individually to wild-type females. Since Dichaete belongs to the third and Bristle to the second linkage groups, the progeny of these crosses was expected to consist of four classes of flies in about equal numbers, namely, (1) wild type, (2) Dichaete, (3) Bristle, and (4) Dichaete Bristle. This is, indeed, what was observed in 117 out of the 121 test crosses. But in 4 test crosses, the classes (2) and (3) proved to be absent, as the following numbers of flies obtained in one of these test crosses show:

wild	Dichaete	Bristle	Bristle Dichaete
106	82

The genes Dichaete and Bristle are completely linked, as though they were located in the same chromosome. The four cultures which showed this apparent linkage were studied further both genetically and cytologically, and they proved to contain translocations involving the second and the third chromosomes.

If a translocation heterozygote is a *Drosophila* female, crossing over will occur between homologous parts of the chromosomes. The linkage be-

tween the genes carried in these chromosomes will no longer be absolute but only partial. It will be strongest, however, between the genes which lie close to the points in the chromosomes at which it had been broken in the process of formation of the translocation (C, D, I, J, C', J', I' , and D' in the scheme on p. 256) and will be the weakest between the genes remote from these points ($A, F, G, L, A', L', G', F'$ in the scheme). The intensity of linkage, and the frequency of recombination, between the genes in translocation heterozygotes permits, therefore, localization of the points of chromosome breakage in terms of the linkage maps of the chromosomes. Cytological examination of the same translocation heterozygotes enables one to correlate the genetically observed changes in the linkage relationships with the state of the chromosomes as observed under a microscope.

For example, in a strain of *Drosophila melanogaster* linkage was observed between genes belonging normally to the third and the fourth linkage groups, indicating a translocation between these chromosomes. A study of recombination disclosed that the third chromosome had been broken between the loci of the genes pink and curled (p and cu , Fig. 98, p. 227). The chromosomes of females heterozygous for this translocation appear as shown in Fig. 116. One of the large V-shaped chromosomes has one of its limbs much shortened; there is only one free, dotlike fourth chromosome; a single rodlike chromosome not present in the normal chromosome complement is found. The interpretation of these cytological findings is as follows: The shortened V-shaped chromosome is the third chromosome which has lost the section corresponding to the $cu-Mg$ interval of the linkage map (Fig. 98); this section is attached to a part of the fourth chromosome, giving rise to the "new" rodlike chromosome. This type of analysis has been carried out for a number of translocations in *D. melanogaster*, in maize, and in a few other species.

Inversion. Soon after the discovery of linkage and recombination in *Drosophila melanogaster*, it was noticed that some strains of this fly contain what was described as "C factors," which reduce or suppress the recombination of genes in a certain chromosome or a part of a chromosome in females heterozygous for such C factors. Sturtevant (1926) found that one of the C factors, which acted as a suppressor of recombination in the right limb of the third chromosome, that is, from the centromere to the right end,



Fig. 116. A chromosomal complement in an individual heterozygous for a translocation between the third and fourth chromosomes in *Drosophila melanogaster*. Note a single dotlike normal fourth chromosome, a hook-shaped fragment of the third chromosome which has lost a section, and a small rodlike chromosome, which is the result of the union of a section of the third chromosome with the dotlike fourth.

specifically, from the loci of the genes *Dfd* and *p* to *ca* and *Mg* (Fig. 98), was an inversion of a section of this chromosome. The reason why recombination is suppressed in inversion heterozygotes will become clear when the cytological effects of inversions are considered.

✓ It may be noted that not only inversions but also heterozygous translocations and large duplications and deficiencies reduce the frequency of crossing over in the chromosomes involved. This is apparently caused by the difficulties which the chromosomes encounter in establishing the meiotic pairing between the leptotene and the pachytene stages. Consider, for example, the situation in a nucleus of a translocation heterozygote, which has the chromosomes *ABCDEF*, *GHIJKL*, *ABCJKL*, *GHIDEF*. Every

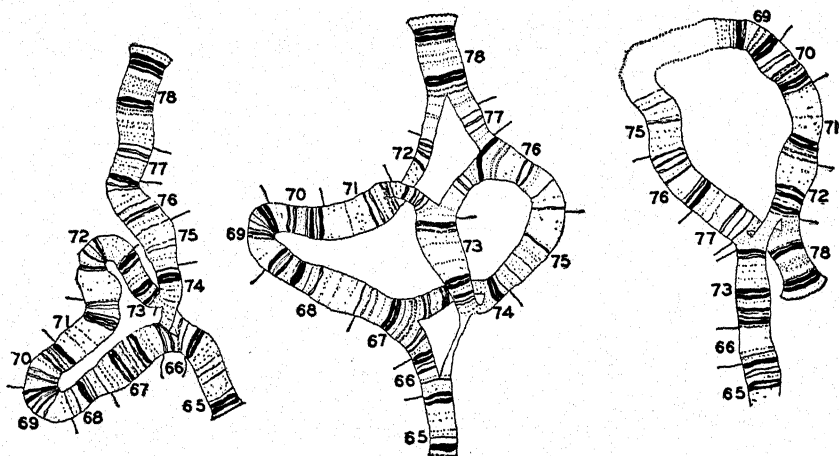


FIG. 117. Inversions in salivary chromosome B^1 of *Drosophila azteca* as revealed by loop formation. (From Dobzhansky and Sokoloff.)

one of these four chromosomes contains parts that are homologous to parts of two other chromosomes in the same nucleus. At the onset of meiosis, when homologous loci begin to attract each other and establish the synaptic association, mechanical pulls and stresses may arise which will delay or prevent the pairing of all homologous chromosome parts, especially of those adjacent to the points of change of linear homology. Disturbance of pairing interferes with the establishment of chiasmata and crossing over.

Suppose that an individual is heterozygous for an inversion, that is, has a chromosome carrying the genes *ABCDEFGH* and a chromosome *AEDCBFGH*. Since homologous loci are paired at meiosis, as well as in the salivary-gland chromosomes, inversion heterozygotes will show, in the cells in which a complete pairing is attained, configurations like those represented in Figs. 117 and 118. Such configurations have been observed at pachytene stage

in chromosomes of maize and in the salivary-gland chromosomes of inversion heterozygotes in several species of *Drosophila*. Since a chromosome once changed by an inversion may undergo another change by another

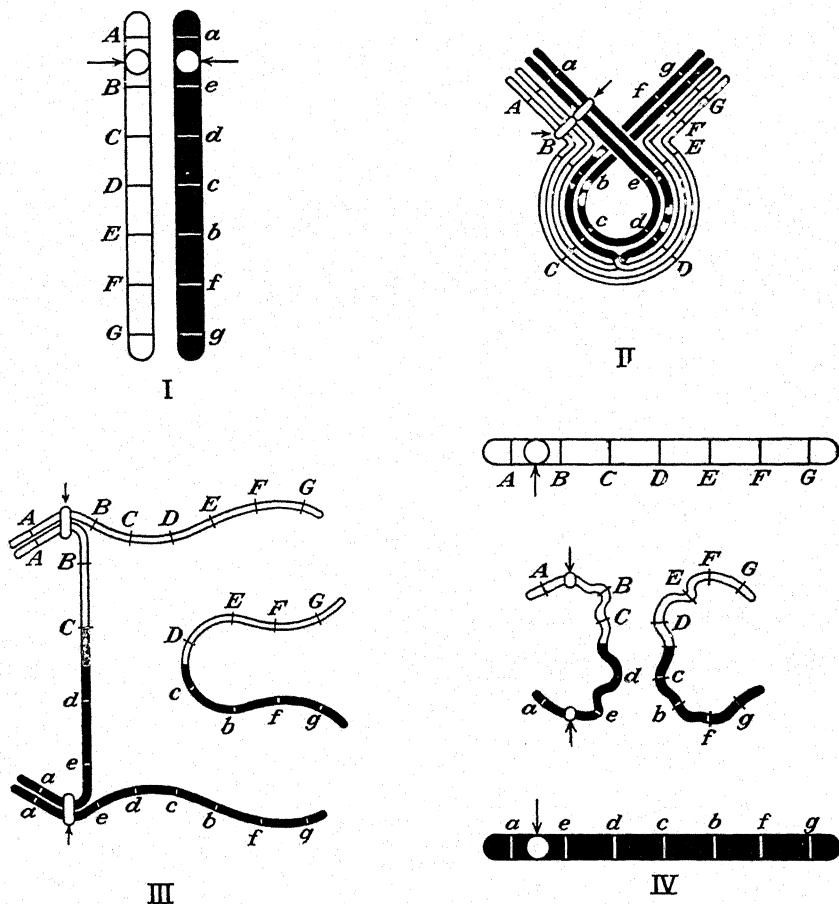


FIG. 118. Crossing over in a heterozygote for a paracentric inversion (not including the centromere). I, two chromosomes differing in a paracentric inversion; II, diplotene stage of meiosis showing a chiasma inside the inverted section; III, anaphase of the first meiotic division, showing a chromatid bridge and an acentric fragment; IV, the outcome of meiosis: two noncrossover chromosomes with normal gene complements, and (in the middle) chromatids with two centromeres and with no centromere (inviable). The centromeres are indicated by arrows.

inversion, even more complex pairing configurations may be observed. An example of a relatively complex one is shown in Fig. 117.

Chiasmata may become established in the paired inverted segments as shown in Figs. 117 and 118. The subsequent fate of the crossover chro-

matids will be different depending upon whether the centromere lies within or outside the inverted section. If the inversion does not include the centromere (*paracentric inversion*), the meiotic anaphase will contain a chromatid which connects the two centromeres, called a *chromatid bridge*,

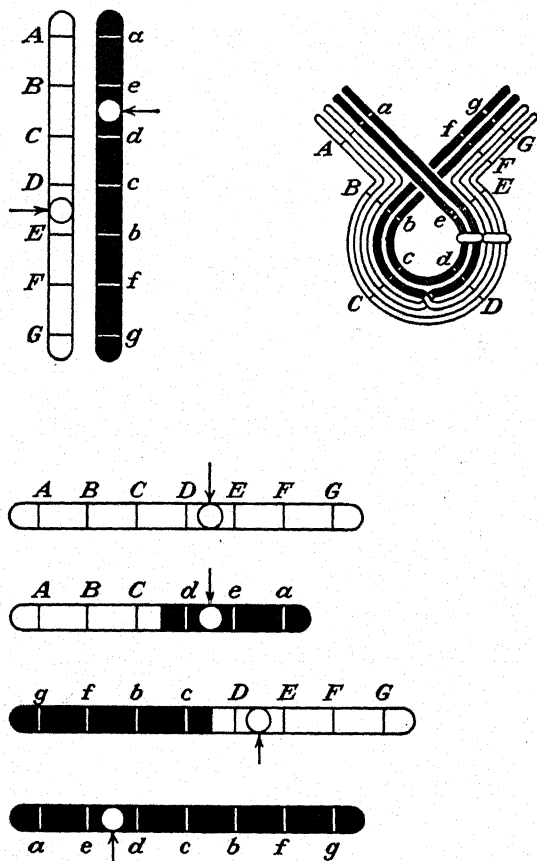


FIG. 119. Crossing over in a heterozygote for a pericentric inversion (including the centromere). I, two chromosomes differing in a pericentric inversion; II, diplotene stage of meiosis, showing a chiasma in the inverted section; III, the four products of meiosis, two of which (the black and the white ones) are noncrossovers and carry normal complements of genes, and the other two (partly black and partly white) are crossovers and carry some genes in excess and are deficient for other genes. The centromeres are indicated by arrows.

and a chromatid lacking a centromere altogether, called an *acentric fragment*, as shown in Fig. 118, III. The formation of chromatid bridges and acentric fragments at the meiotic divisions has been observed by McClintock in maize and by Darlington, Stebbins, and others in a series of other plants. The presence of such bridges and fragments furnishes

a cytological method of detection of inversions which is used in organisms in which pachytene chromosomes or salivary-gland chromosomes are either absent or unfavorable for investigation. Now, neither the chromatid bridges nor the acentric fragments behave normally in cell divisions and are eventually lost. The only viable products of meiosis in paracentric inversion heterozygotes are the chromatids which underwent no crossing over within the inverted section (see Fig. 118). Sturtevant and Beadle (1936) have assumed that, in oögenesis, one of these noncrossover chromatids becomes included in the egg nucleus, while the other chromosomes are eliminated in the polar bodies. This would explain the suppression of recombination observed in inversion heterozygotes.

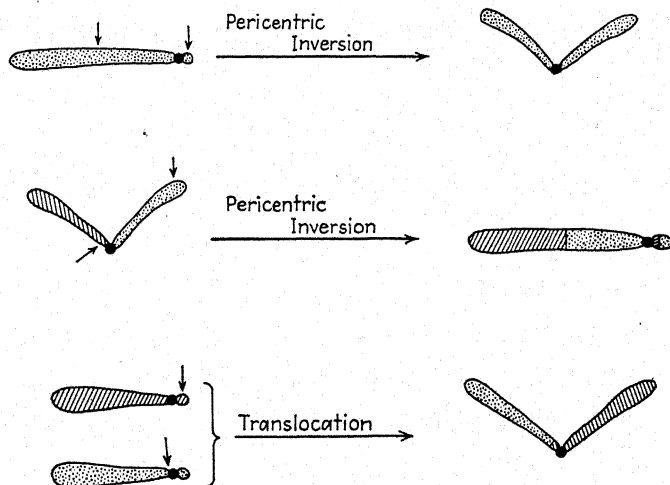


FIG. 120. Changes in form and in number of chromosomes owing to pericentric inversions and translocations. Centromeres are shown as black circles; places at which chromosomes are broken to give rise to inversions and translocations are shown by arrows.

Some inversions include the locus of the centromere and are called *pericentric inversions* (Fig. 119). Pericentric inversions sometimes lead to changes in the appearance of a chromosome at mitosis in somatic cells. For example, a chromosome with a median centromere usually appears as a more or less equal-armed V-shaped body (like the second and third chromosomes in *Drosophila melanogaster*); if the two breaks giving rise to a pericentric inversion occur at different distances from the centromere in the two arms, the resulting chromosome will have a more or less subterminal centromere and will appear hook-shaped or rod-shaped (Fig. 120). Conversely, a pericentric inversion in a hook-shaped or rod-shaped chromosome may transform it into a V-shaped one with more or less equal arms. Such changes in the shape of chromosomes have been observed in laboratory cultures of *Drosophila*, and they are known to occur in nature as well.

Crossing over in a pericentric inversion does not result in production of chromatid bridges and acentric fragments at the *first* metaphase of meiosis. It does result, however, in formation of chromosomes having duplications for some genes and deficiencies for others (Fig. 119). Gametes which receive such chromosomes do not as a rule form viable zygotes. Recombination is therefore lowered in heterozygotes for pericentric inversions.

Permanence of the Centromere. Studies on chromosomal aberrations have emphasized the importance of the centromere, or the place where the chromosome is attached to the spindle in the meiotic and mitotic divisions.

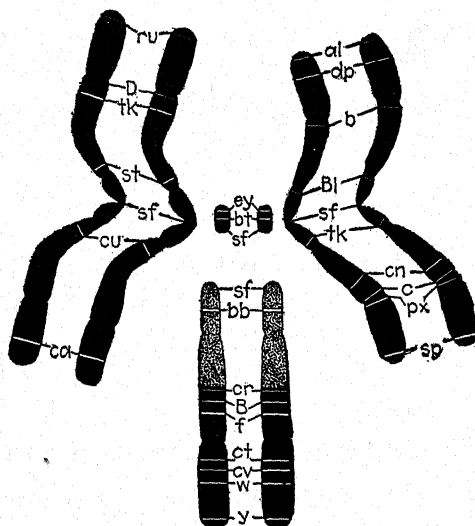


FIG. 121. Cytological maps of the chromosomes of *Drosophila melanogaster*, showing the approximate location of various genes and of the spindle fibers (sf). The inert region of the X chromosome is represented by the stippled portion of the rod-shaped chromosomes. The longer V-shaped chromosomes (left) are the third chromosomes; the shorter V-shaped chromosomes (right) are the second chromosomes; the smallest pair, the fourth chromosomes. (From Dobzhansky.)

Drosophila, maize, and most other organisms important in genetic research have one and only one centromere per chromosome. The acentric fragments, that is, those chromosomes lacking a centromere formed as a result of crossing over in paracentric inversions (p. 259), do not behave normally in cell division, are usually left out of the nucleus in the cytoplasm, and eventually die. A similar fate of acentric fragments is observed also in cells in which chromosomes are fragmented by X rays or other means. Dicentric chromosomes, that is, chromosomes with two centromeres, are also inviable, because they are frequently ruptured during mitosis. Such chromosomes have been seen in irradiated cells, and dicentric chromatids occur at meiosis in paracentric inversion. Among numerous translocations obtained in *Drosophila* and in maize the altered chromosomes invariably

have one and only one centromere each, although translocations forming dicentric and acentric chromosomes doubtless arise and are eliminated.

The position of the centromere among the gene loci on linkage maps of chromosomes of *Drosophila melanogaster* (*sp-a*, Fig. 98, p. 227) was inferred, even before studies on chromosomal aberrations permitted it to be established beyond reasonable doubt, from observations on interference and crossing over in different chromosomes. We know that the occurrence of crossing over at some point in a chromosome diminishes the likelihood of another crossover taking place in the neighborhood of this point. There is, however, no interference across the centromeres in the V-shaped second and third chromosomes; the two limbs of a V-shaped chromosome behave

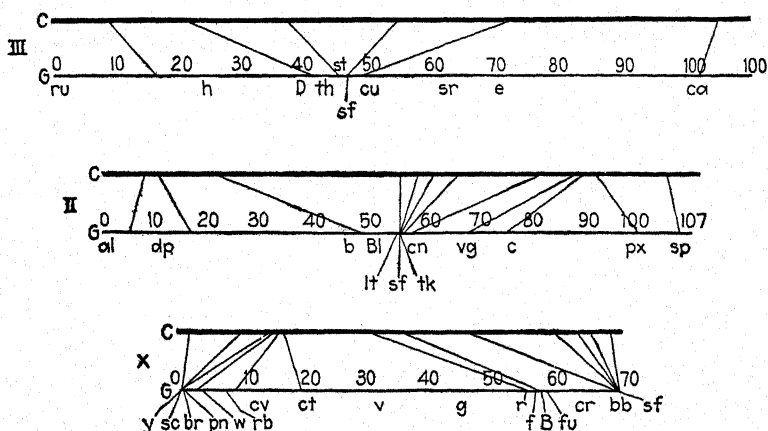


FIG. 122. Comparison of the genetic and cytological maps of the third (III), second (II), and X chromosomes (X) of *Drosophila melanogaster*. C, the cytological map; G, the genetic map. Figures indicate the genetic distances in map units. (From Dobzhansky.)

quite independently in crossing over. Similarly, heterozygosity for a paracentric inversion in one limb of such a chromosome does not suppress crossing over in the other.

Cytological Maps. Correlated genetical and cytological studies on chromosomal aberrations have permitted construction of *cytological maps* of chromosomes, which show the location of various genes in terms of the microscopically visible chromosomes. Cytological maps of metaphase chromosomes of *Drosophila melanogaster* are shown in Fig. 121. The positions of some of the genes in this fly have also been determined in the chromosomes as seen in the salivary-gland cells (Fig. 110, p. 249). Rough cytological maps of the chromosomes of *D. pseudoobscura* have been made by Tan. E. G. Anderson and others have succeeded in making such maps for some chromosomes in maize.

The linear orders of the genes shown by cytological maps and by the linkage (genetic) maps are invariably the same (Figs. 98 and 121, p. 227).

The work on cytological maps has therefore wholly confirmed the theory of linear arrangement of genes in chromosomes, put forward originally on the basis of studies on recombination of traits in crosses (cf. p. 215).

The relative distances between the genes on cytological and linkage maps do not always correspond (Fig. 122). The discrepancies are greatest in the vicinity of the centromeres (marked *sf* in Fig. 98), where one crossover unit corresponds to a relatively much greater distance on the physical chromosome than in other regions. Conversely, the genes lying rather close to each other in the middle of each limb of the second and third chromosomes appear relatively much farther apart on the linkage maps. Taken as a whole, the linkage maps represent the physical distances between genes in a chromosome as though seen in an uneven mirror; some parts of chromosomes are relatively compressed, while other parts are overextended.

The origin of these discrepancies in the spacing of genes on cytological and on linkage maps is not far to seek. It should be recalled that the "distances" between the genes on linkage maps are measured by the frequencies of recombination, in per cent, observed in hybridization experiments involving these genes. Hence, if in some parts of a chromosome, for example in the vicinity of the centromere, crossing over takes place relatively rarely, this part will appear foreshortened on the linkage map. Conversely, crossing over is evidently frequent in the part of the chromosome lying about midway between the centromere and the free end, and genes lying in this part appear far apart on the linkage map. A glance at the linkage maps of *Drosophila melanogaster* (Fig. 98) will show that some parts of the "chromosomes" are crowded with known genes, while others appear rather devoid of genes. The distribution of the known genes on the cytological maps, with the important exceptions described below, is more uniform than it is on linkage maps.

Euchromatin and Heterochromatin. The most striking discrepancy between the cytological and the linkage maps in *Drosophila melanogaster* (Figs. 121 and 122) is that approximately one-third of the length of the X chromosome contains only a single known gene, bobbed, while more than 100 other genes lie in the remaining two-thirds. Now, bobbed is the only gene in the X chromosome which has an allele also in the Y chromosome. The Y chromosome, although it is larger in size than the X in dividing cells, is known to contain, apart from bobbed, only some factors which are necessary for the fertility of males. It appears, then, that about one-third of the X chromosome and the whole of the Y chromosome consist of some material which is different from that composing the remainder of the chromosomes.

Even before the above facts were disclosed, cytologists had found that in their preparations some chromosomes and chromosome parts remain

CHROMOSOME ABERRATION AND CYTOLOGICAL MAPS

darkly staining in interphase nuclei, or begin to stain in the prophase, while other chromosomes stain only faintly or not at all. The precociously staining chromosome sections are said to be *heterochromatic*, while the remainder of the chromosomes are *euchromatic*. Heitz, who studied this phenomenon in various plants and animals, made the observation (1934) that the Y chromosome, about a third of the X chromosome, and smaller sections near the centromeres in the second and third chromosomes of *Drosophila melanogaster* consist of heterochromatin. These are, however, just the parts of the chromosomes which contain fewer genes per unit length than other, or euchromatic, chromosome parts. Heterochromatic sections, known as "knobs" occur also in the chromosomes of maize, and here they appear to be devoid of known gene loci.

In *Drosophila*, the euchromatic and heterochromatic sections behave very differently in the salivary-gland chromosomes. The giant size of the chromosomes in the salivary-gland cells is due very largely to enormous expansion of the euchromatic chromosome parts, compared with their sizes in chromosomes of other cells. The heterochromatic parts are relatively short, and they do not show the clear alternation of the darkly staining and light disks characteristic of the euchromatin. Furthermore, the heterochromatic parts of all the chromosomes have a tendency to associate with one another, and to form the chromocenter, from which radiate the wormlike euchromatic chromosome strands. In *Drosophila*, and in some other organisms, heterochromatin shows a tendency to be concentrated near the centromeres of some or of all chromosomes, although shorter heterochromatic sections seem to be present also in other parts intercalated among euchromatic ones.

Imp. ✓ Cytological Demonstration of Crossing Over. Convincing evidence of the association of genes with chromosomes is further provided by the proof that, where there is an interchange of material between two homologous chromosomes, there is also an interchange of genes by crossing over. This has been shown in a number of cases, but Stern's demonstration in *Drosophila* is simple and complete. The essential feature of this demonstration is the use of strains in which it is possible to distinguish the two members of a pair of chromosomes from each other and from normal chromosomes of the same set. This is sometimes possible when as a result of *translocation* a portion of one chromosome has broken away and become attached to another, thus constituting a visibly different configuration. By this means Stern was able to obtain a strain of *Drosophila* in which a portion of a Y chromosome had through translocation become attached to the end of one of the X chromosomes, forming a somewhat L-shaped body, easily distinguished from a normal X. In another strain one of the X chromosomes had been broken into two approximately equal parts. The

terminal portion containing the centromere remained in its normal position. The other portion was translocated to one of the small IV chromosomes. The strain was viable, since the entire material of the X chromosome was present although in two separate pieces. By crossing these strains and producing females in which one X chromosome showed one of these translocations and the other X the other, it was possible to distinguish both the X chromosomes in the same individual from each other and from the autosomes.

Stern now succeeded in obtaining such females as were heterozygous for two sex-linked mutations located in the upper end of the X chromosome: carnation, *cr*, an eye-color mutant, and Bar, *B*, causing a narrowing of the eye. Carnation is recessive and Bar dominant. Such a fly therefore has eyes of wild-type (red) color and Bar shape, with the genotype $\frac{cr\ B}{++}$. It was known from the way in which the stock had been made up that the *cr* and *B* genes were in the upper half of the broken X chromosome and that their two normal alleles were in the X bearing the translocated Y portion. Such a female was bred to a male having both the recessive genes (*cr* and the wild-type allele of Bar) in its X chromosome. The offspring of such a cross of double heterozygote by double recessive could be classified by inspection into four groups, as in any case of linkage. The females alone were studied. Of these there were two noncrossover classes (carnation Bar and normal) and two crossover classes (carnation with normal shape and Bar with normal color). These may be represented as follows:

Noncrossovers		Crossovers	
$\frac{cr\ B}{cr\ +}$	$\frac{+ +}{cr\ +}$	$\frac{cr\ +}{cr\ +}$	$\frac{+ B}{cr\ +}$

The X chromosomes of these four classes of females were then studied. One of each pair, coming from the male parent, should evidently be normal. Its mate, coming from the female, should be distinguishable by its abnormal character and might be expected to show the effect of any cytological crossing over which had occurred in the maturation of the eggs. The genetic and cytological results are shown in Fig. 123. It is evident that in the two classes of genetical noncrossovers the maternal X is as it was in the mother, either broken into two or entire and provided with the translocated fragment. In the genetical crossovers, however, the maternal X has evidently resulted from crossing over, since in the Bar, noncarnation flies the fragment of the Y is now attached to the upper half of the divided X, and in the carnation, non-Bar flies an apparently normal chromosome is present. These are the results which should obtain if there had been an

interchange between the two X chromosomes of the mother at a point near the upper end of the X and between the locations of the *cr* and *B* genes. Of the F_1 female flies, 364 were tested, and in all but 5 (and these pre-

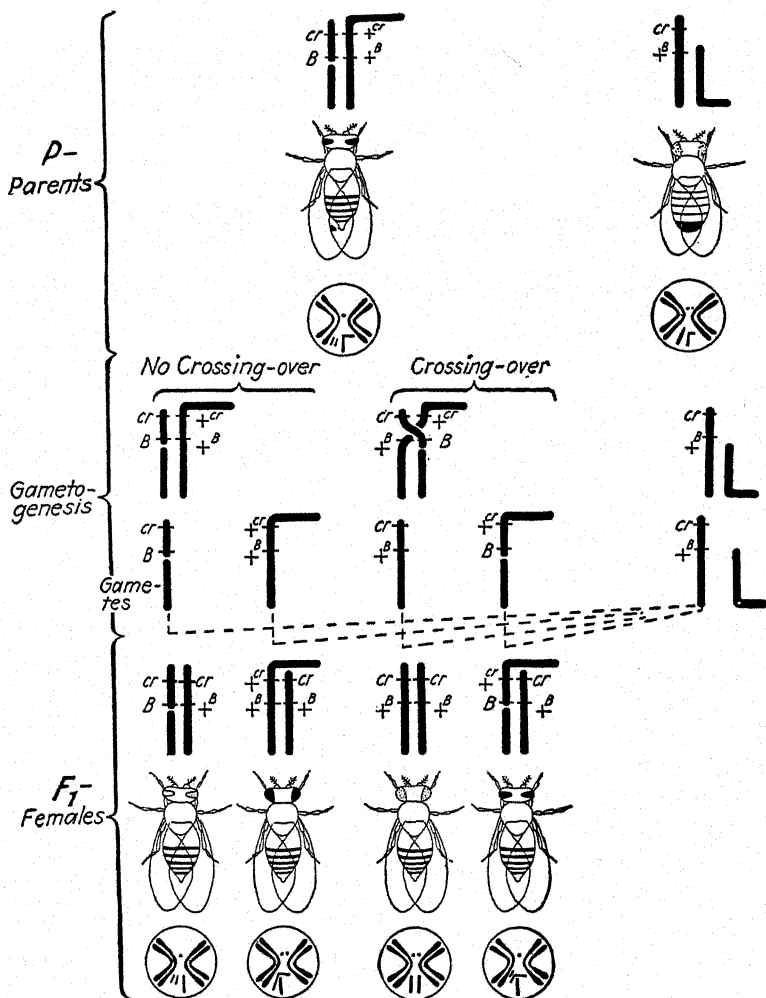


FIG. 123. Diagram of Stern's experiment demonstrating crossing over cytologically. See text for details. (From Stern.)

sumably the result of experimental error) there was a complete correspondence between the genetic and the cytological facts. In other words, genetic crossing over was proved to be accompanied by cytological crossing over, an actual exchange of material between homologous chromosomes.

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PROBLEMS

279. In maize assume that strain 1, which is homozygous for the recessive endosperm characters *a*, *b*, *c*, *d*, *e*, *f*, *g*, and *h*, is pollinated by pollen from strain 2, which is homozygous for the dominant alleles *A*, *B*, *C*, *D*, *E*, *F*, *G*, and *H*, but that the

pollen has been subjected to irradiation before being placed on the styles of strain 1. If some of the resulting kernels are phenotypically *AbcdEFGH*, how would you explain this result?

280. Strain 1 with genes *A, B, C, D*, and *E*, in the same chromosome and known to be arranged in that order, is crossed with strain 2, homozygous for the recessive alleles of all of these genes. The F_1 crossed back on *abcde* is found to produce only four types of gametes: *ABCDE*, *ABCDe*, *abcdE*, and *abcde*. Explain these facts.

281. Strain 1 with genes *A, B, C, D, E, F, G, H*, and *I*, in the same chromosome and known to be arranged in that order, is crossed with strain 2, which is known to possess all the recessive alleles of these genes. Backcrosses show that in the F_1 there is crossing over between *A-B*, *G-H*, and *H-I* but never between *B-C*, *C-D*, *D-E*, *E-F*, or *F-G*. Explain these facts, and map the chromosome, for these genes, as it occurs in strains 1 and 2.

282. In the case of semisterile maize (see Fig. 112) all the genes in each complete set of chromosomes (1-2 and 3-4, for example) are, of course, linked, just as though they were in one chromosome. Rhoades studied cases of double crossing over in such a system, where one crossover was in one arm and the other in the opposite one (as in arms 2 and 3 in Fig. 112), and found that there was no interference, coincidence being 1.0. Explain this difference from the usual behavior of double crossovers.

283. In maize, strain I and strain II differ by a single segmental interchange. I is genetically *aa* and II is *AA*. The F_1 from a cross between them is semisterile and *Aa*. When this is crossed back on strain I, the following offspring are produced: 35 per cent normal, *a*; 35 per cent semisterile, *A*; 15 per cent normal, *A*; 15 per cent semisterile, *a*. Where, with reference to locus *a*, is the translocation point?

284. Strain I, above, is also homozygous for gene *bb* and II for *BB*, so that the semisterile hybrid is *Bb*. When this, as before, is crossed back to strain I, the following offspring are produced: 45 per cent normal, *b*; 45 per cent semisterile, *B*; 5 per cent normal, *B*; 5 per cent semisterile, *b*. It is known that, in strain I, *a* and *b* are in the same chromosome and between 25 and 30 units apart. For strain I, map the chromosome in which *a* and *b* occur, showing the position of these genes and of the translocation point.

285. In strain I, genes *c* and *d* are in the chromosome which later undergoes segmental interchange with that containing *a* and *b*, thus forming strain II. In strain II, *d* is now found to be linked with *b*, and *c* with *a*. By use of data similar to that presented in the two preceding problems, it can be shown that *c* gives 10 per cent crossing over with the translocation point and *d* gives 5 per cent. Make a diagram of the four chromosomes involved in the translocation between strains I and II as they appear at synapsis in the semisterile hybrid between these strains, showing the location of the four genes and the two translocation points.

286. A recessive gene plexus (px), in the second chromosome of *Drosophila melanogaster*, always shows pseudodominance when combined with the dominant mutant Minute-1 ($M-l$) but not with $M-l^2$, which behaves as an allele of $M-l$. Suggest an interpretation of this, together with breeding experiments and other observations by which your hypothesis could be tested.

287. Notch female, red-eyed, by wild-type (normal-winged), white-eyed male gives white-eyed, Notch and red-eyed, normal offspring. If one of their white-eyed, Notch offspring is mated with a red-eyed male, what will be the appearance of their offspring?

288. In *Drosophila*, male flies heterozygous for eyeless dominant (EyD) and stubble (Sb) were test crossed individually to wild-type females. Some males gave four classes of offspring (wild-type, stubble, eyeless, and eyeless stubble) while others gave only wild-type and eyeless stubble. Suggest a hypothesis to explain this and a cytological test of your hypothesis.

CHAPTER XI

MUTATION

Children resemble their parents because the genes of descendants are derived from those of their ancestors. Genes arise only from genes. Whenever a chromosome divides, all the genes of which it is composed reproduce themselves. Gene reproduction means that a gene engenders a copy of itself out of materials present in the cell, and since this gene copying may go on indefinitely, heredity, in the last analysis, is due to the accuracy of gene reproduction.

The resemblance between children and parents and between ancestral and descendant populations, however, is never complete. This variability is partly due to environmental modifications, but a part of it is hereditary or genotypic. The commonest cause of hereditary variability in sexually reproducing organisms is *gene recombination*. Every gamete which is produced contains only one-half of the genes and chromosomes of the parent; and different gametes contain different combinations of the parental genes. If two parents are each heterozygous for a recessive gene, a part of their progeny may be homozygous for that gene and may be phenotypically distinct from the parents. The new phenotypes which arise from segregation and recombination, such as the examples of reversion given in Chapter V, are due not to anything new or changed in the hereditary mechanism itself but to reassortment of the existing elements.

A part of the hereditary variability is caused, however, by alterations in the hereditary materials, or *mutations*. Exact though the process of gene reproduction is, occasionally one copy differs from the original and the modified gene then reproduces its changed structure just as faithfully as the ancestral gene did. This is *gene mutation*. Again, the chromosomes, which are assemblages of genes, occasionally undergo alterations in which some or all of the genes may be lost, may be present in excess, or may change their relative positions with respect to other genes. Such *chromosomal mutations* or *aberrations* have been reviewed in Chapter X. Whole sets of chromosomes may be reduplicated (polyploidy) or lost (haploidy). When only certain chromosomes are multiplied or lost, *heteroploids* are produced. Sections of chromosomes with the genes contained therein may be present in excess of normal (duplication) or may suffer loss (deficiency). Different chromosomes may exchange sections (translocation), or blocks

of genes may alter their position within a chromosome (inversion). Duplications, deficiencies, translocations, and inversions inevitably break the association of some genes which normally lie next to each other in the linear series within a chromosome and establish new associations between genes which were not in contact in the original chromosomes. Such changes of position of genes within a chromosome sometimes change the functioning of these genes (*position effect*, Chap. XVIII). Position effects are a class of changes which are in a sense intermediate between gene mutations and chromosomal mutations.

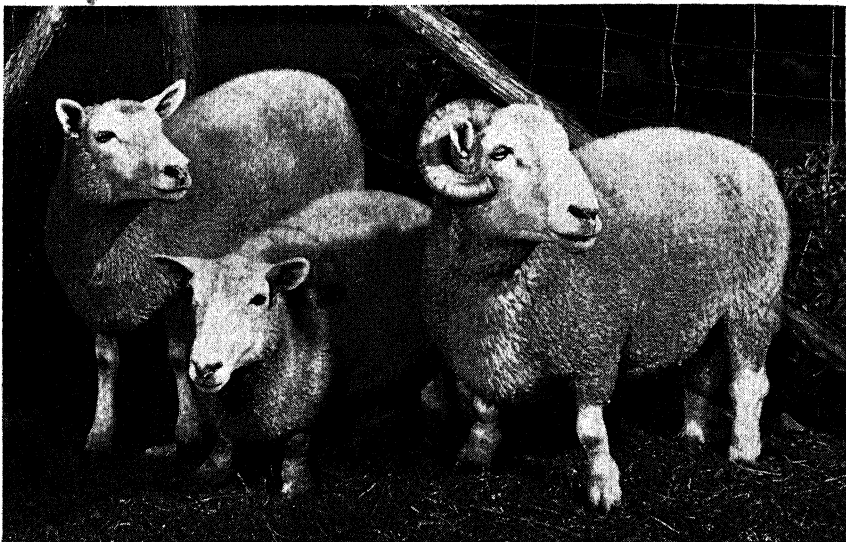


FIG. 124. The Ancon (short-legged) mutation in sheep (ewe in center, ram at right) compared with a normal ewe at left. (Photograph from *Life Magazine*, courtesy of Storrs Agricultural Experiment Station.)

Historical. Acceptance of Darwin's theory of evolution naturally made the problem of the origin of hereditary changes very important, for without such changes evolution is scarcely possible. Darwin himself devoted much attention to this problem. He had collected instances of the sudden appearance of new hereditary types recorded by plant and animal breeders and referred to by them as "sports." These suddenly appearing sports, most of which have to be classed as mutations in modern terminology, have often been points of departure for the creation of new varieties or breeds. Thus, in the latter part of the eighteenth century, for example, there appeared in the flock of Seth Wright, a New England farmer, a male lamb with remarkably short, bowed legs. Wright reared this lamb and bred from it, thereby originating the Ancon breed of sheep, so short-legged that

they could not jump over an ordinary stone wall. This breed became extinct about eighty years ago, but some fifty years later another short-legged lamb appeared suddenly in the flock of a Norwegian farmer, representing probably a new appearance of the same mutation. From this, a new strain of short-legged sheep has been bred (Fig. 124).

In the same way, hornless mutant individuals have appeared in almost all breeds of horned cattle, and hornless strains of these breeds have been developed from them. Pacing horses, double-toed cats, "mule-footed" swine, albino rats, and many other new, distinct, and true-breeding types have appeared as mutations. A mutation in man is shown in Figs. 125 and 126.

The same type of variation has appeared in plants. The Shirley poppy, the dwarf "Cupid" sweet pea, and the dwarf, cut-leaved, double-flowered, and white-flowered races of many plants have each descended from a single plant of this type which appeared under cultivation. The new, or *mutant*, character has arisen suddenly, has bred true from the beginning, and has thus given rise to a new and distinct variety.

Most of these mutations in plants arose from seed, but in some instances the mutant character was found to be confined to a single branch. Such a branch, when artificially propagated, remains true to its new type. Many horticultural varieties, especially those with variegated foliage, have arisen from such mutations, or "bud sports."

Continuous and Discontinuous Variability. Darwin emphasized the wide divergence from the normal type produced by sports, but nevertheless he ascribed to them a very minor role in evolution because he regarded them as too apt to produce monstrosities instead of viable new types. Darwin based his theory of evolution on the supposition that natural selection favors the survival and the spread of the best-adapted products of "fluctuating variability," a term applied to the ubiquitous small differences in size, color, shape, and other traits found among individuals in any population of any species. It was only in the first quarter of the present century, long after Darwin's death, that this fluctuating variability, in so far as it is hereditary at all, was shown to be due to the

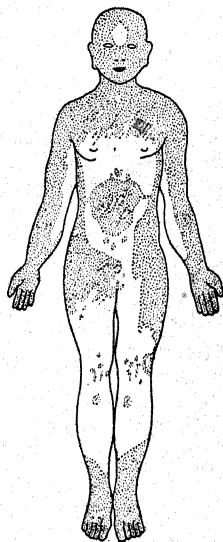


FIG. 125. Sketch of a man showing the type of piebald spotting which arose by mutation in a Norwegian family. The white "blaze" or forelock on the forehead and white hairs in irregular patterns on the ventral body surfaces are constant features of the character. The same type of spotting has appeared independently in unrelated families in other parts of the world. (After Sundför.)

segregation of genes with very small effects. Such gene differences, which have been discussed in Chapter VI, are basically similar to the larger differences which, at their origin, give rise to the sudden and striking sports.

The opposite view, that large discontinuous variations are the chief sources of evolutionary change, was, however, advocated by Bateson (1894), Korjinsky (1899), and especially by Hugo de Vries, who, in his "mutation theory" (1901 to 1903) introduced the term *mutation* for large discrete changes in the genotype. De Vries supported his views by exten-

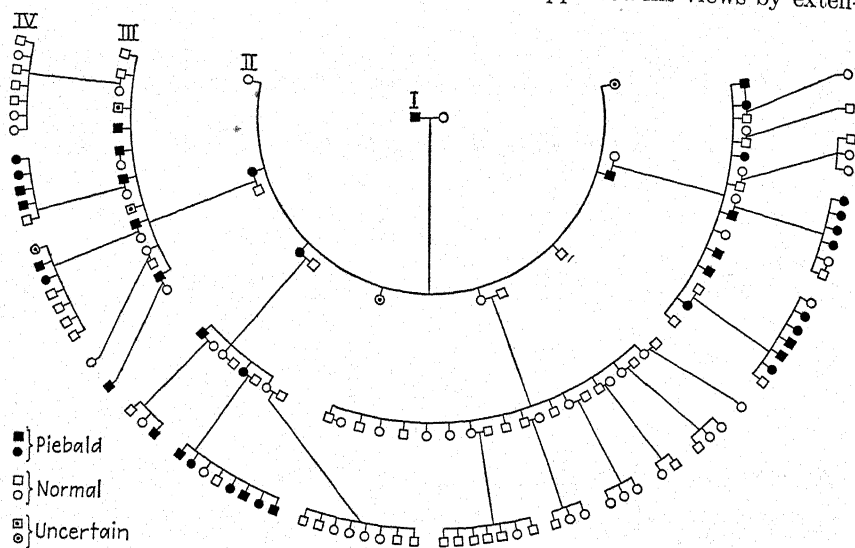


FIG. 126. Pedigree chart of dominant piebald spotting in a Norwegian family. The character first appeared, probably by mutation, in one of the gametes which gave rise to ♂ I-1, center. (After Sundför.)

sive observations on mutations in the evening primrose, *Oenothera Lamarckiana*.

De Vries' insistence that the process of the origin of sudden and drastic changes, mutations, is basically different from the origin of Darwin's small or fluctuating variations proved to be unfounded. The studies of Morgan and his school on mutations in *Drosophila*, begun in 1909, showed that mutations of all magnitudes occur in that fly. The differences between a mutant and the parental form may sometimes be large, but they may also be so minute that refined statistical methods are needed to detect the occurrence of the mutation at all. Mutations of every conceivable intermediate extent also occur. The mutation process is the source of all hereditary variability and is the mainspring of evolution, for it supplies the raw materials from which evolutionary changes can be built.

Mutations in *Oenothera*. Lamarck's evening primrose, *Oenothera Lamarckiana*, is a native of America, but De Vries found it growing as a weed in Holland and was struck by its unusual variability. In De Vries' garden it produced a number of more or less striking mutant types each season (Fig. 127). One type, called *gigas*, differed from the parental form by its large size; another, *nanella*, was a dwarf; still others differed in color, size, or shape of various parts. Now, what De Vries described as mutations proved to be changes of several quite different kinds. Thus, the mutant *gigas* proved to have 28 chromosomes instead of the 14 present in the parental form. It is a tetraploid, and its origin was due to the doubling up of the entire chromosome complement. Some of the mutants were *trisomics* (cf. p. 246); they carried 15 chromosomes, because some one chromosome of the normal complement was present three times. Perhaps only two of De Vries' mutants represented gene mutations. Others, including *nanella*, arose by a process which can be understood only in the light of the peculiar genetic properties of *Oenothera Lamarckiana* described on pages 300 to 303. Thus it turned out that the species on which the observations were made that led to the mutation theory has since been proved to be a rather peculiar organism and its "mutations" a mixture of many different types of hereditary changes, some of them quite rare.

Mutation in *Drosophila* and Other Organisms. A classical example of mutation was the sudden appearance in 1909 in a true-breeding strain of red-eyed *Drosophila melanogaster* of a single white-eyed individual which, when bred, behaved as a sex-linked recessive (p. 180). All the available data indicate that this mutation involved a change in a specific point in the X chromosome which can be localized on the linkage maps and the cytological maps of this chromosome. Here a wild-type gene had suddenly changed in such a way that processes of development which before had resulted in red eye color now resulted in eyes without pigment. This change was a permanent one since the white-eyed strain derived from the mutant bred true thereafter.

Subsequently, many hundreds of *gene mutations*, or *point mutations*, have been detected among the at least 10 million unchanged specimens of this fly which have been examined by competent observers. Whenever tested, each mutation resulted in a specific and stable genotypic difference. Mutations never arise gradually, increasing in intensity in several consecutive generations. A full-fledged mutant appears usually as a single individual among unchanged representatives of a strain.

The same kind of evidence has been obtained from several other species of *Drosophila*, from maize, snapdragons, rodents, bread molds, man, and other plant and animal species which have been observed in large numbers. Mutations have been found also in bacteria, in bacteriophages, and in viruses, but in most of these organisms no Mendelian segregation of traits



FIG. 127. *Oenothera Lamarckiana*, the evening primrose (center) with two mutant types which arose from it; *O. gigas*, the giant form (right), and *O. nanella*, the dwarf (left). (From B. M. Davis.)

in the offspring of hybrids is usually observed because regular sexual reproduction in these lowest types of life appears to be rare or absent. There is little doubt that the differentiating traits which Mendel studied in peas had arisen as gene mutations. In fact, mutation is the only known method by which allelic differences between genes can arise.

All kinds of morphological and physiological traits, both external and internal, arise by mutation. In *Drosophila*, mutations are known to affect coloration of the eyes and the body, wings, bristles, legs, size of the body and its parts, sexual characters, fertility, longevity, and behavior (reaction to light and gravity). Some mutations may produce *homeosis*, that is, transformation of one organ into another. Thus, mutations are known that transform the antennae of *Drosophila* into legs, balancers into a second pair of wings, or sucking mouth parts into parts resembling those of some lower insects. In the bread mold *Neurospora* numerous mutations have been found which change the food requirements of the fungus and block certain biochemical reactions which are fundamental in cellular metabolism (p. 282). Bacterial mutants may lose virulence or acquire it, become resistant or susceptible to antibiotics such as penicillin or sulfa drugs or to attacks by bacteriophages, may acquire or lose the ability to grow on food media with or without certain nutritive constituents, or may affect the appearance of the colonies of the bacteria grown on agar plates.

Mutations are usually named according to the traits which they change and which appear most striking to the observer. Thus, the mutants "white" and "yellow" in *Drosophila* differ from normal flies by having white, instead of red, eyes and a yellow, instead of a brown, body, respectively. This convention of naming mutants should not be taken to indicate that a mutant differs from the ancestral form always in one character only or even less that the gene white is a gene "for eye color" and without any other effects. The great difference in eye color between the mutant white and the normal fly is indeed most conspicuous, but the mutant differs from wild type also in having a transparent instead of yellow testicular envelope, a spermatheca of a different shape, a lessened viability, longevity, and fertility, and other pleiotropic effects. A careful study would probably disclose that many or perhaps even all genes are pleiotropic (*cf.* p. 79).

To De Vries and his immediate followers, the term "mutation" meant a striking alteration of the phenotype. Some mutations are indeed drastic enough to upset the fundamental processes of embryonic or postembryonic development and thus to act as lethals. Mutations which are most useful in genetic experiments demonstrating Mendelian segregation, linkage, crossing over, and related phenomena and which are for this reason mentioned most frequently in textbooks produce changes sufficiently conspicuous to

be recognized at sight. But, as shown by Johannsen, some mutations are so slight that they can be detected only by the most competent and well-trained observers. Timoféeff-Ressovsky and Kerkis showed by means of specially devised experiments that such small mutations constitute, in fact, a majority of all mutations that occur. Of course, small and large mutations are not different categories, since mutations form an uninterrupted spectrum, beginning with the most drastic ones and ending with those which are at the lower limit of detectability. All mutant individuals that arise, whether from small or large mutations, always belong to the same biological species to which the ancestral form belongs. This is true as well for those mutants in which alterations have occurred in traits which distinguish species, genera, and even higher taxonomic categories. For example, the order of flies, *Diptera*, differs from other insect orders among other things in possessing only one pair of wings. The mutant bithorax (*tetraptera*), in which the balancers became a second pair of wings, is still not only a fly but a member of the genus *Drosophila* and the species *melanogaster*.

Stages at Which Mutations Occur. A mutation may occur at any stage in the development of the organism. If a mutation takes place in one of the gametes produced in a gonad, a single individual in the progeny will contain a mutant gene which, if dominant, will appear in a single one of the progeny. On the other hand, a mutation in one of the two daughter chromosomes formed at the first mitotic division in the fertilized egg will give rise to an individual about half of whose body cells will be normal and the other half will carry the mutation. If the mutation is dominant, such an individual will be a *mosaic*, or a *fractional mutant*. Mosaics are formed in multicellular organisms if a mutation takes place during development, the fraction of the body which shows the mutant traits being smaller the later in development the mutation has occurred. A mutation may affect only a single somatic cell, which, as in color variation in the endosperm of maize or in the epidermis of flowers or leaves, shows a specific difference from surrounding cells. In the larkspur, *Delphinium*, Demerec has found that somatic mutation in flower color occurs at rather early stages and at late stages of the development of the flower with higher frequency than in intervening stages (Fig. 128). Mutations in cells ancestral to the gametes such as spermatogonia may affect several or many gametes, resulting in the appearance of clusters of mutant individuals in the progeny.

When a group of somatic cells seems to be genotypically different from other cells in the same individual, a *somatic mutation* may be suspected. Therefore, one of the working hypotheses of the origin of tumors is that these neoplastic growths arise by somatic mutation. A cancer-producing mutation, on this hypothesis, would change the properties of certain cells

in such a way that their multiplication and growth are no longer under the normal control prevailing in the organism. Recent work, showing that certain chemical substances which induce cancers in some body parts in higher animals also increase the frequency of mutations, may be considered as lending support to the above hypothesis. Much further work will, however, be required to establish whether or not neoplastic growths arise owing to somatic mutations.

Quantitative Studies on Mutations. [Rapid progress in the study of mutation became possible only after Muller, in the 1920's, developed techniques for the quantitative estimation of the frequency of mutations.] Indeed, without such techniques the detection of mutations involves a



FIG. 128. Flowers of Delphinium showing somatic mutations from rose to purple. Large areas result from early mutation, small ones from later mutation. (From Demerec.)

large "personal equation," since some observers overlook many of the lesser mutations which attract the notice of other observers. Furthermore, since mutations arise in one gene at a time, a dominant mutation may be detected at once, while a recessive one may be present in heterozygous condition in a strain for many generations before it chances to become homozygous and thus apparent to the eye.

Estimates of mutation frequency, or "rate," depend very much on the kind of observation or measurement which is applied. If we use a microscope to detect changes, of course we have the opportunity to detect many more differences than the unaided eye can distinguish. But if we confine our attention to lethal mutations, then we have only to detect the presence or absence of a whole category, such as males or females, among the descendants of a parent in whom the occurrence of mutation is being tested. Muller's first success in measuring mutation frequency was due to his

invention of an accurate method for detecting recessive lethal mutations in *Drosophila*. Similarly, Stadler's use of special methods for detecting mutations in maize, barley, and other plants permitted the detection and measurement of mutation frequency in plants (p. 293).

Detection of Lethal Mutations. One of the most useful methods is the "*ClB* method" of detection of recessive mutants in the X chromosome of *Drosophila melanogaster* (Fig. 129). The *ClB* X chromosome contains the dominant gene Bar (*B*, narrow eye), a recessive lethal (*l*, no expression in heterozygotes), and an inversion (*C*) which eliminates crossing over. A female carrying a *ClB* chromosome has Bar eyes. A male getting this chromosome dies on account of the lethal. Now, suppose that a *ClB* female is crossed to a normal male. As shown in Fig. 129, half of the male

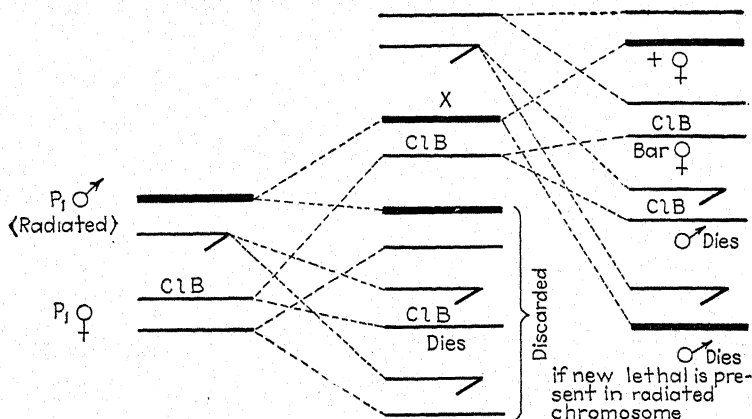


FIG. 129. The *ClB* method for detecting lethal mutations in the X chromosome in *Drosophila melanogaster*.

progeny will die and the sex ratio will be about 2 ♀♀:1 ♂ among the survivors. Half of the females will carry the *ClB* chromosome, and they can be recognized because of their Bar eyes. If these females are tested singly, by crossing each of them to a male in a separate culture, the following results may be obtained: If the X chromosome of the original male which was carried in the spermatozoon producing a given Bar-eyed female was normal, the offspring will again consist of females and males in a ratio approaching 2:1. If, however, a spermatozoon contained an X chromosome in which a recessive lethal had arisen by mutation, the Bar-eyed female will carry a lethal in her *ClB* chromosome and another lethal in her second X, received from her father. In the offspring of such a female, half of the males die because of the lethal in the *ClB* chromosome, and the other half die because of the newly arisen lethal in the other X chromosome. The result is a progeny consisting only of daughters and no sons. Again,

if the spermatozoon contains an X chromosome with a newly arisen recessive mutation producing, for example, white eyes, the surviving sons of the *CIB* female will have white eyes. If enough *CIB* females are thus tested, one can determine what proportion of the spermatozoa of a normal male or of a male treated with X rays, chemicals, or other agents contain newly arisen sex-linked mutations of various types. Thus it has been determined that between 10 and 20 per 10,000 spermatozoa of an untreated male of *Drosophila melanogaster* (between .1 and .2 per cent) contain X chromosomes with newly arisen sex-linked lethal mutants.

Detection of Visible Mutations. The "Attached-X" Method (Fig. 130). Another method has been devised especially for the detection of visible mutations in the X chromosome of *Drosophila*. This utilized the peculiar

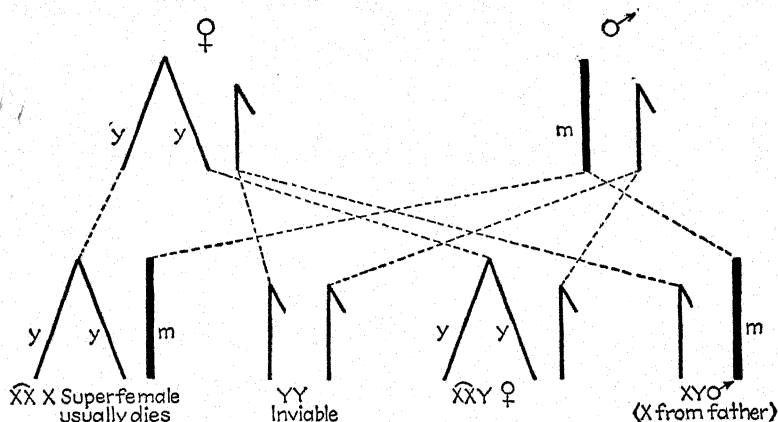


FIG. 130. The "attached-X" method for detecting visible (and lethal) mutations in the X-chromosome in *Drosophila melanogaster*.

stock discovered by L. V. Morgan in which the X chromosomes show 100 per cent nondisjunction in the female and in which it has been shown cytologically that the females contain two X chromosomes attached to each other and in addition a Y chromosome (Chap. VIII). When crossed with normal males, the daughters, XXY, receive their X chromosomes from the mother and the Y chromosome from the father, while the sons get their Y chromosome from the mother and their X from the father—just the reverse of the usual method. This obviously provides the opportunity for any new recessive mutation arising in the X chromosome of the father to appear immediately in the sons.

In practice, the X chromosomes of both parents are "marked" by recessive genes, for example, y = yellow body in both maternal X's, m = miniature wing in the paternal X. The P_1 males are irradiated and bred to attached-X females. Some sons exhibit directly the effects of new sex-linked genes which have arisen as a result of the treatment of the father.

Techniques of detection of mutations, similar in principle to the *CIB* method, have been devised for autosomes of *Drosophila melanogaster* and for other species the genetics of which is sufficiently well known to permit "tagging" of chromosomes by "marking genes" and inversions.

Detection of Nutritional Deficiency Mutants in Neurospora. Beadle and Tatum devised a simple and ingenious method for detection of muta-

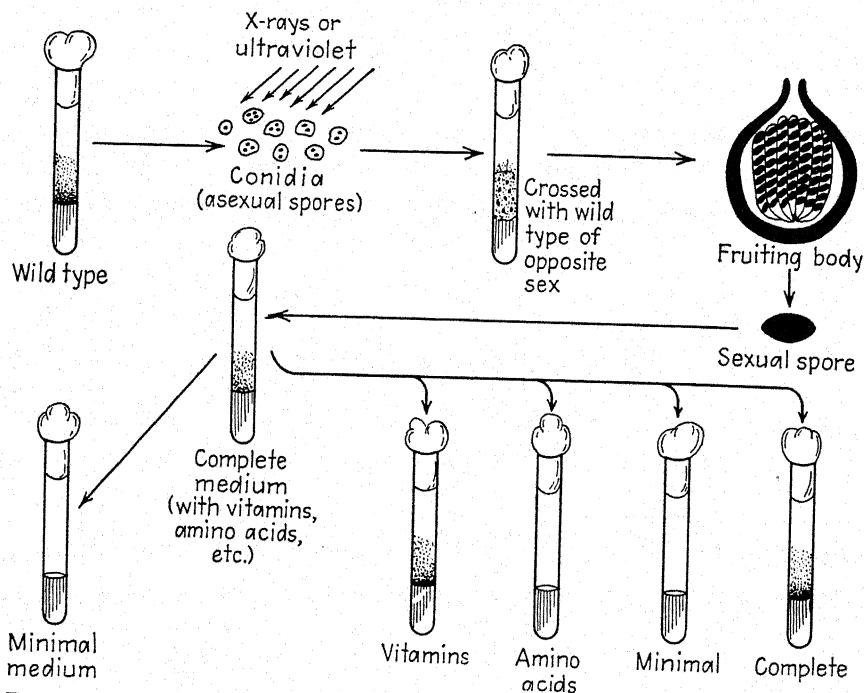


FIG. 131. Method of detection of biochemical mutants in *Neurospora*. The mutant in the above case fails to grow on minimal medium, and on minimal medium enriched by a mixture of amino acids, but does grow when vitamins are added. It lacks ability to synthesize one of the vitamins, cf. Fig. 132. (From Beadle, *Science in Progress*, by permission of Yale University Press.)

tional changes involving nutritional requirements in the pink bread mold *Neurospora*. This fungus can be grown on a "minimal" culture medium, which consists of sugar, a nitrogen source such as ammonium nitrate and tartrate, some inorganic acids and salts, and a single vitamin, biotin. The standard or "complete" medium on which the mold is cultured contains, however, many more organic compounds, vitamins, amino acids, and other substances. Ascospores from a *Neurospora* culture, untreated or treated by a physical or chemical agent, are placed singly in tubes with complete medium and allowed to produce mycelial growths. Then parts of the

mold from each tube are transferred to tubes with the minimal medium. If growth continues normally, no change in the minimal nutritional requirements has taken place. Sometimes, however, no growth is obtained on the minimal medium; and this indicates that a mutation blocking some physiological process essential for growth on this medium has occurred. Just what process is blocked in a given mutant can be determined by testing the mutant strain on minimal media to which various supplemental substances have been added. For example, if a mutant strain fails to grow

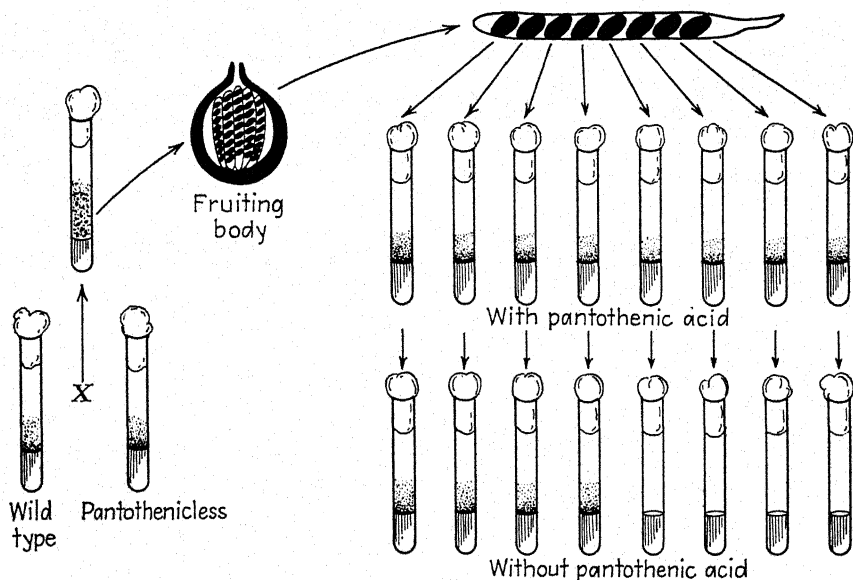


FIG. 132. The method of determining the inheritance of a biochemical mutant type in *Neurospora*. Conidia are transferred from medium with the test substance (pantothenic acid in above case) to medium without the test substance. Segregation of the mutant type occurs in the manner shown. (From Beadle, *Science in Progress*, by permission of Yale University Press.)

on the minimal medium but does grow on a medium to which pantothenic acid has been added, the conclusion is that the mutation interferes with some reaction essential for the synthesis of this compound in the growing mold (Figs. 131 and 132).

In experiments of Beadle, Tatum, and collaborators, reported in 1945, some 60,000 spores derived from treatments with X rays, ultraviolet rays, and other agents were tested by the above method. Among these, mutations of at least 100 genes affecting nutritional requirements were observed. These mutants offer valuable material for studies on gene-controlled biochemical reactions which take place in the body of the fungus (see Chap.

XV). Variants of the technique of detection of nutritional-deficiency mutants developed for *Neurospora* have been successfully used for similar work in microorganisms, particularly in bacteria. Studies on genetically determined metabolic reactions constitute a rapidly growing field being actively developed at present.

Mutations in Bacteria. The small size and rapid reproduction of bacteria, viruses, and other microorganisms permit obtaining millions and even billions of individuals in a single culture. This makes these lower forms of life exceptionally favorable for studies on mutational changes, since such studies with higher organisms are frequently handicapped by the rarity of mutations, which would make it necessary to raise prohibitively large numbers of experimental plants or animals. Furthermore, bacteriologists have known for a long time that changes do occur in bacterial cultures. Pathogenic organisms may lose their virulence when cultured on artificial media and may "regain" it by a passage through a susceptible host. Bacteria cultured on certain media may be "trained" to grow on other media which were originally unsuitable for them, etc. But the very ease and regularity with which such changes are obtained made it uncertain whether genotype changes or merely phenotypic modifications were involved, and many bacteriologists felt inclined to interpret their observations according to Lamarckian ideas. The recent work of Luria, Delbrück, Demerec, Fano, and others has placed the problem of mutation in bacteria on a new basis.

If a suspension of bacteriophage is added to a culture of susceptible bacteria, bacteriophage particles (which are too small to be seen in an ordinary microscope but can be photographed by the electron microscope) multiply in the bacterial cells and cause the death of the cells. But an occasional bacterial cell survives despite the presence of many particles of phage, and from such a survivor a new strain of bacteria may be obtained which is now completely resistant to the phage. Such a bacterial strain resistant to a certain phage may remain nevertheless fully susceptible to other phage lines which are known to attack the bacterial species in question. However, bacteria resistant to any one line may usually be obtained by exposing susceptible bacterial cultures to the proper phage and picking up the few surviving bacteria (Fig. 133). Similarly, a bacterial strain which had become resistant to one phage line may acquire resistance to one or more other lines by exposure to the latter and isolation of the resistant survivors.

These facts may be interpreted in one of two ways. First one may suppose that mutations resistant to various kinds of bacteriophage occur in a bacterial strain from time to time, regardless of whether this strain is or is not exposed to the phages. In the absence of bacteriophages, such resistant mutants have no advantage over the ancestral susceptible form or

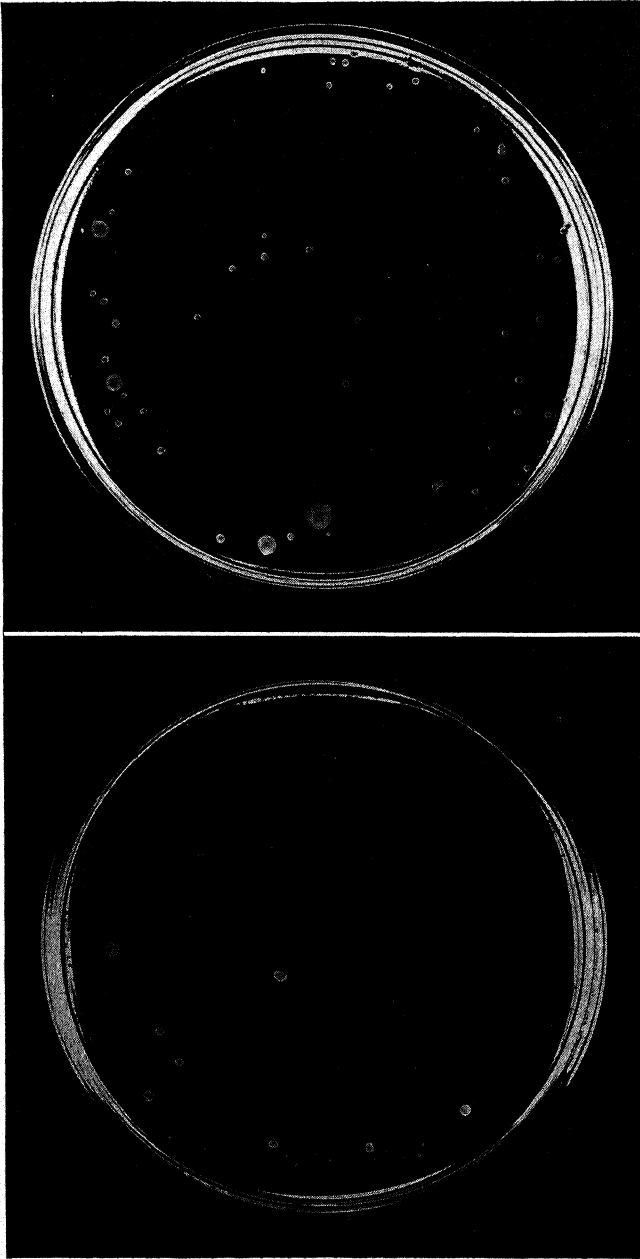


FIG. 133. Mutant colonies of *Escherichia coli*; left, large and small colonies; right, large and tiny colonies. The bacteria are growing on Petri dishes coated with one strain of bacteriophage. Each colony type is characteristic of one clone of phage-resistant bacteria arising by mutation.

have even a slight disadvantage compared with it. But when exposed to a given bacteriophage, all susceptible cells succumb, and only the few available mutants resistant to the phage survive and give rise to resistant strains. Second one might suppose that resistance is somehow induced in a few bacterial individuals by contact with bacteriophage. It is possible to test the validity of these two interpretations by a simple statistical method. If mutation to resistance takes place regardless of exposure to bacteriophage, the number of resistant mutants per culture will vary greatly depending upon when the mutation happens to occur. Their number will be large if a mutant appears early during the multiplication of the bacteria and produces many mutant cells by division, but only a small number of resistant cells will be present if the mutation takes place shortly before the bacteriophage is applied. It can be shown mathematically that the variance (*cf.* p. 139) of the number of resistant cells in different cultures will be larger than the mean number of such cells per culture. On the other hand, if the resistant bacteria appear only when the bacterial culture is exposed to the bacteriophage, the number of resistant cells in different cultures raised under uniform environmental conditions will be subject only to the variation due to sampling errors. Mathematically this will result in the variance being about as large as the mean number of resistant cells per culture. Tests have shown that the variance in most experiments is much greater than the mean.

It may therefore be concluded that resistance to bacteriophages is produced by mutations which occur in the bacteria regardless of exposure to the phages.

When a large number of phage particles are added to a culture of bacteria resistant to the particular phage line, a few particles may prove to be able to attack the bacteria and from them a new phage line may be isolated which overcomes the bacteria resistant to the original line. Luria has demonstrated that what is involved in the appearance of new bacteriophage lines is mutation in the phage, which again occurs regardless of whether the bacteria are or are not resistant to the original phage line. A similar demonstration has been given by Demerec for the development of resistance in certain bacteria to penicillin, by Luria for resistance to sulfa drugs, and by Witkin for resistance to the killing action of ultraviolet light and X rays. The penicillin resistance is interesting, particularly because it has been found that a bacterial strain originally killed by relatively very small concentrations of penicillin in the culture medium acquires resistance gradually in many generations, being able to withstand greater and greater concentrations of penicillin in the media to which it is transferred. The gradualness of this process, which appeared to be difficult to reconcile with the mutation theory, proved to be due to the fact that highly resistant

bacterial strains arise by accumulation of several successive mutations, each of which taken separately induces only relatively slight gains in the degree of resistance.

However, it is not an invariable rule that "adaptation" phenomena in microorganisms involve mutational changes in the genotype. Spiegelman and Lindegren found that some strains of yeasts acquire enzymes necessary to ferment certain carbohydrates when placed on culture media containing the latter, although when cultured on media with other carbohydrates the same yeasts do not contain these enzymes. A yeast strain which has acquired an enzyme through being placed on the proper culture medium may lose this enzyme again if transferred back to a medium in which the respective carbohydrate is absent. What is involved in these changes is evidently a genotype which reacts to the presence of certain carbohydrates in the environment by producing an enzyme and to the absence of the same carbohydrates by suppressing the production of this enzyme. A very interesting detail is that other strains of the same species of yeast are unable to produce a similar enzyme regardless of the medium on which they are cultured, unless a mutation takes place. The two kinds of strains, those which do and those which do not "adapt" on exposure to the carbohydrate, seem to differ in a single gene.

Frequency of Mutation. Although it is often said that spontaneous mutation is a rare event, this is true only in the sense that mutational changes in any one gene are, as a rule, infrequent. The aggregate frequency of mutations in all genes is not low; Timoféeff-Ressovsky and Muller have estimated that, in *Drosophila*, between 5 and 10 per cent of individuals carry a newly arisen mutation in every generation. In the snapdragon, *Antirrhinum majus*, Baur estimated the over-all mutation frequency to be as high as 5 to 7 per cent. The insuperable difficulty in determining this figure more exactly is due to the fact that most mutations produce changes so minute that they are usually overlooked, despite the modern techniques for the detection of mutations.

More reliable estimates exist for certain special classes of mutations, such as lethals. Measurements of mutation rates have disclosed, however, a very interesting and important fact, namely, that strains of the same species may show heritable differences in mutability. According to Demerec and to Dubinin, although in most strains of *Drosophila melanogaster* between .1 and .2 per cent of X chromosomes acquire a new recessive lethal in every generation, in some strains the frequency is as high as 1 per cent. In one case, the high mutability has been shown to be due to the presence of a special gene carried in the second chromosome the action of which consists in increasing the mutability of the genotype. Mampell has found a gene increasing the general mutability in *Drosophila persimilis*.

Different genes likewise vary greatly in their mutability. In maize Stadler made a careful study of the frequency of mutation of seven genes. His data (Table XXV) show that some loci (such as *R*) are relatively mutable, while others (such as *Wx*) are extremely stable. In *Drosophila* a similar situation probably exists.

In *Drosophila* many spontaneous mutations at such loci as white, cut, forked, ebony, and vestigial have now been observed, forming long series of multiple alleles and including recurrent mutations to the same allele. Many mutations at these loci have also been observed following irradiation; but at other loci only one or a few mutations have been found. There are known, for example, at least 14 alleles of the gene for white eyes in *Drosophila melanogaster*; in several of the rodents 3 to 7 alleles at the albino locus are known; and other genes which have been observed just as continuously have mutated but once to produce a single pair of alleles.

TABLE XXV. THE FREQUENCY OF CHANGES IN SEVEN GENES OF MAIZE
(After Stadler)

Gene	Gametes tested	Number of mutations	Average per 1 million gametes
<i>R</i>	554,786	273	492
<i>I</i>	265,391	28	106
<i>Pr</i>	647,102	7	11
<i>Su</i>	1,678,736	4	2.4
<i>Y</i>	1,745,280	4	2.2
<i>Sh</i>	2,469,285	3	1.2
<i>Wx</i>	1,503,744	0	0

Mutation is a reversible process. A gene normally present in a species (the wild-type allele) may change to a new mutant allele (direct mutation), and the mutant allele may change back to the wild type (reverse mutation). Timoféeff-Ressovsky has shown that, for some genes in *Drosophila melanogaster* such as white, direct mutation takes place much more frequently than the reverse one, while for other genes, such as forked, mutation in both directions is about equally frequent.

In the colon bacteria, *Escherichia coli*, mutations to resistance to various strains of bacteriophages take place, according to Demerec and Fano, with frequencies ranging from 1 in 10^6 to 1 in 10^8 individuals per generation.

Mutation in Man. Although methods of measuring mutation frequency like those employed above cannot be applied to man, other considerations indicate that gene mutations like those found in lower animals and plants are by no means rare in the human species. The best evidence comes from genes which are lethal or semilethal but which yet maintain their frequency in spite of the obvious selection against them. For example, hemophiliacs,

which are always males (*cf.* p. 183), usually die before they can pass on their defective gene to their offspring and have less than a quarter as many children as their normal brothers. Haldane has pointed out that since about one-third of all hemophilic genes are in the X chromosomes of males and are thus exposed to the risk of extinction, while two-thirds are in the female X chromosomes, where they are hidden by the normal allele, we can suppose that almost one-third of the hemophilia genes will be extinguished in each generation. The average life of any single hemophilia gene would thus be not much over three generations. Since the frequency of sex-linked hemophilia remains roughly constant, it must be kept up through the origin of new hemophilia genes by mutation. Two other kinds of direct observations confirm this. One is that in many human pedigrees which are quite completely known for several generations, like that which includes the descendants of Queen Victoria, hemophilia appears suddenly at one point, as though a mutation had occurred in one X chromosome of a female, in this case, as it happened, in the queen herself. The other fact is that the sex-linked hemophilia which appears in different families often shows certain clinical differences, as though different mutant alleles of the hemophilia locus had arisen by separate mutations in the separate families.

The frequency of mutation at this locus was estimated by Haldane on the basis of the incidence of hemophilia in London to be between 5×10^{-5} and 1×10^{-5} per X chromosome per generation. Andreassen, on the basis of more complete data from Denmark, places it at 2×10^{-5} , or 1 per 50,000, as the upper limit of mutation frequency at this locus.

Estimates of the mutation rate of other loci based on the incidence of diseases due to single gene mutations are, according to Haldane:

Epiloia or tuberous sclerosis (Gunther and Penrose).....	1.6×10^{-5} (1/60,000)
Chondrodystrophy (dominant) (Mørch).....	9.6×10^{-5} (1/10,000)
Aniridia (Möllenbach).....	2.0×10^{-5} to 1×10^{-5}

These are rather high rates as compared with average rates for single loci in maize or *Drosophila*. Nevertheless one other more reliable estimate, based on the frequency of a human gene which could be detected in heterozygotes, is still higher. The gene in question, as studied by Neel and Valentine, produces a fatal anemia, known as thalassemia major or Mediterranean anemia, in homozygotes, which thus never transmit the gene, but a mild and harmless blood disturbance known as thalassemia minor in heterozygotes. It has been estimated that in order to maintain this gene in equilibrium frequency in the population (*cf.* p. 319) a mutation frequency of at least 4×10^{-4} (1/2,500) is required. Since the gene is practically confined to persons of Greek or Italian descent, other genes in these persons may be responsible for the high frequency.

If we apply the average rate for the less mutable loci, such as hemophilia,

say 1/50,000, and estimate that no less than 1,000 loci are subject to independent mutation, then the over-all mutation rate in man would be at least 2 per cent, that is, about 2 chromosomes per 100 per generation would contain a newly arisen mutant gene, which means that any egg or sperm, with its 24 chromosomes, has a chance of about .5 per cent to have a new mutant.

It is of course difficult to establish either the fact or the time of origin by mutation of recessive visible mutations in man since in the absence of close inbreeding these may be transmitted through heterozygotes for many generations. In the case of clear-cut dominants, however, the origin of a new gene may be indicated by its sudden appearance in single ancestral individuals in unrelated pedigrees in different parts of the world. Thus a dominant gene causing white forelock and other white spotting on the body (Fig. 126, p. 126) has appeared in at least 12 different unrelated pedigrees in different countries and races, in each case appearing first in a single ancestor. The difficulties of estimating frequencies from such observations are obvious, but there is little doubt that, in the mutability of individual loci and in total mutability, man is comparable with the animal and plant species which have been studied.

Almost nothing is known of chromosome aberrations in man, although in one pedigree a six-linked gene, color blindness, has been transmitted from *mother to all daughters* and to no sons for five generations. This has been explained by Roberts as due to attachment, or 100 per cent nondisjunction, of X chromosomes as in the case of *Drosophila* described on page 189.

✓ **Mutable Genes.** At one end of the range of gene stability are genes which, like the *Wx* gene in maize (Table XXV), mutate very rarely. At the other end are the so-called mutable genes, some of which mutate so frequently that every individual carrying them is a mosaic of mutated and unmutated tissues. In addition, the frequency of mutation in different tissues may be different. For example, the gene for variegation in the pericarp of maize, studied by Emerson, mutates only in somatic but not in the germinal tissues. The parts of the seed derived from the cells in which the mutation had taken place show the dominant self-colored condition, indicating that the recessive gene (variegation) has mutated back to its dominant allele again. Emerson also found evidence of another gene in maize which increased the frequency of these reverse mutations. Demerec has discovered several mutable genes in *Drosophila* and in *Delphinium* (Fig. 128, p. 279). In *Drosophila virilis* one strain of miniature-winged flies, known as miniature alpha (miniature is a recessive sex-linked gene as in *D. melanogaster*), regularly produces 5 to 10 per cent of wild-type (long-winged) flies as well as some mosaics in which patches of wild-type tissue

appear in the miniature wing. Demerec has shown that the appearance of the wild-type flies is due to reverse mutation of a miniature gene to its wild-type allele in the germ cells, whereas the mosaics are due to the same type of mutation in the somatic cells. He has, moreover, demonstrated the inheritance of several genes that stimulate the mutability of the miniature gene. One of these, *M*, behaves as a dominant and when present in miniature alpha flies may result in the production of over 75 per cent of wild-type progeny. The mutability of the same miniature gene in the somatic cells may be unaffected.

An even more striking case has been studied by Rhoades in maize. The dominant allele of a gene, *A*₁, gives rise to a purple coloration in various parts of the plant, while the recessive allele, *a*₁, gives green plants. Under normal conditions both alleles mutate very infrequently. But in plants which carry the dominant gene *Dt* (Dotted, located in a different linkage group from that which carries the locus *A*₁—*a*₁), the gene *a*₁ mutates to *A*₁ so frequently that the plant has green leaves streaked with purple and seeds that are speckled with purple dots. The gene *Dt* has no other known effects apart from modifying the mutability of the gene *a*₁. The mutability of the allele *A*₁ is not affected by *Dt*.

Induction of Mutations by Radiation. Most mutations that have been observed arose "spontaneously," that is, in animals or plants not known to have been exposed to any mutation-inducing treatment. A single mutant individual is usually found among large numbers of unchanged representatives of a strain, and in the mutant individual, as a rule, only a single locus in one chromosome is found to have been altered (see, however, chromosomal aberrations, pp. 246 and 247). Now, saying that a natural phenomenon arises "spontaneously" of course amounts to an admission of ignorance of the real causes of the occurrence. For many years all attempts to induce mutations artificially proved unavailing, until in 1927 Muller, who had developed methods for the quantitative study of the mutation frequencies in *Drosophila* (p. 280), found that the frequency of mutations in the progeny of parents treated with X rays is much greater than the spontaneous frequency. This epoch-making discovery was immediately confirmed by Stadler, who had been working independently on X-ray induced mutations in barley, and was soon extended to a variety of plants, animals, and microorganisms.

Using the *ClB* method (see p. 280), Muller obtained sex-linked lethals, semilethals, and mutations causing visible changes, the numbers of which are shown in Table XXVI. (The treatment designated *t*₄ involved twice as much X radiation as that designated *t*₂).

Thus a total of at least 138 new lethal mutations occurred following X radiation of the grandfather. A few "visible" mutations, that is, external

changes shown to be due to mutation, were also observed incidentally, but their frequency was not accurately measured in this experiment. The frequency of mutation varied with the X-ray dosage, those treated for 48 minutes producing nearly twice as many mutations as those treated 24 minutes.

In another experiment, in which the attached-X method was used to detect the sex-linked visible mutations, Muller obtained the results shown in Table XXVII.

TABLE XXVI

Treatment	Number of fertile F ₂ cultures	New mutations		
		Lethals	Semilethals	Visibles
Untreated.....	198	0	0	0
X ray <i>t</i> ₂	676	49	4	1+
X ray <i>t</i> ₄	772	89	12	3+

As many as 147 visible mutants were observed among the 2,640 tested X chromosomes (every son of an attached-X female carries an X chromosome derived from the father).

Stadler irradiated barley seeds with X rays. Barley provides good material for studying the effect of radiation on mutation frequency since, when planted at sufficient distance, each plant produces several tillers and each tiller bears a terminal flower and seeds. Each tiller arises from a separate primordium in the seed, and a mutation occurring in one primor-

TABLE XXVII

Treatment	Total F ₁ ♂♂	Visible mutants
<i>P</i> ₁ ♂ X-rayed <i>t</i> ₂	1,490	61
<i>P</i> ₁ ♂ X-rayed <i>t</i> ₄	1,150	86

dium affects only a single head; if the mutation is recessive, its effects will appear only when the gametes produced by this head unite in self-fertilization and produce another generation of plants. In practice, normal barley seeds in which primordia have separated are irradiated and planted, and the self-fertilized seeds on each head of the resulting plant are harvested and planted separately.

If a recessive mutation has occurred in the cell from which the entire head is derived, all cells in this head will be heterozygous for it and about one-fourth of the seedlings produced by self-fertilization will show it. If,

as often happens, only part of the head is derived from the cell in which the mutation occurred, less than one-fourth of the offspring will show the change. Other head progenies from the same parent plant will all be normal, proving that the mutation had not occurred prior to the treatment, for then it should appear in the offspring of all the heads.

The correctness of these assumptions can be tested in later generations for in the progeny of a head that segregates $\frac{3}{4}$ normal: $\frac{1}{4}$ mutant, two-thirds of the normal individuals should be heterozygous for the mutation

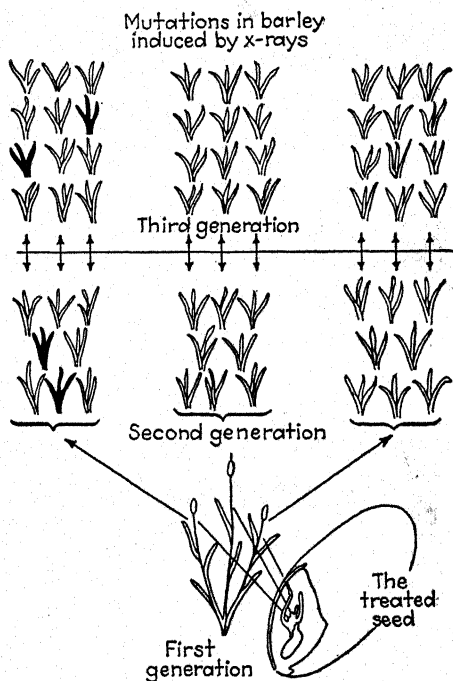


FIG. 134. Method for testing the effect of X rays on the induction of mutations in barley. (From Stadler.)

and should segregate for it when self-fertilized. This was found to be the case (Fig. 134). Since observations have been confined chiefly to seedling characters, it has been possible to observe large numbers of head progenies. In experiments of this type some 800 seedling mutations were recorded. Most of them affect chlorophyll characters (albinism, striping, etc), although many other kinds of mutants have been found (Fig. 135).

In general, these new mutant characters are similar to those which appear in barley under natural conditions, but they are much more frequent in the progeny of irradiated seeds.

✓ **Analysis of the Effects of Radiation on Gene Mutation.** Since 1927, mutations have been induced by X rays in many kinds of organisms, from viruses, bacteria, and protozoa to higher plants and animals. X rays of all wave lengths are effective, from so-called "soft" ones to the "hardest" ones like those emitted by radioactive materials (gamma rays). Mutations are also produced by neutron bombardments. Ultraviolet rays are effective, while visible light and longer wave lengths are not. Mutations



FIG. 135. Induced mutation in barley. Right, normal barley plant; left, "vine," a mutant which arose after application of X rays. (From Stadler.)

are induced only in the cells exposed to the radiations and not in other body cells. For example, if the body of a fly is irradiated but its abdomen, which contains the gonads, is shielded from the radiation by a plate of lead, the frequency of mutations in the gametes is no higher than in nonirradiated control flies. Conversely, if the abdomen is irradiated while the rest of the body is protected, the frequency of mutations in the gametes is as high as when the whole fly is treated. Ultraviolet rays, which, in contrast to X

rays, have a low penetrating power in living tissues, induce no mutations if an adult fly is treated, because the rays are almost entirely absorbed in the superficial tissues of the body and do not reach the gonads. But the mutagenic effect is easily demonstrable if the ultraviolet is applied to pollen grains of maize, to spores of *Neurospora*, or to bacterial cells, which are sufficiently small to be penetrated by the rays.

The frequency of mutations induced by X rays is directly proportional to the amount of the radiation expressed in roentgens (Roentgen units), which are a physical measure estimating essentially the number of ionizations produced by the passage of the rays through matter (Fig. 136). X

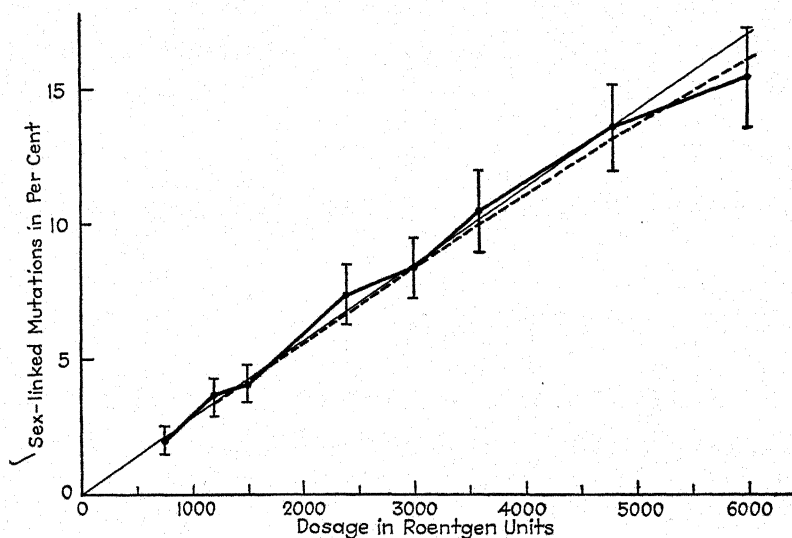


FIG. 136. The proportionality between the frequency of sex-linked mutations and the radiation dosages applied, in *Drosophila melanogaster*. (After Timoféeff-Ressovsky.)

rays of different wave lengths produce equal numbers of mutations, provided that the amounts of the rays measured in roentgens are alike. Furthermore, a given amount of X rays has the same effect regardless of whether it is administered to the organism in a short time from a powerful source of the rays or in a longer time from a weak source. Continuous treatments have the same effects as interrupted ones, provided that the total numbers of roentgens administered are the same. The mutagenic effects of X radiation are, hence, cumulative ones. This fact is important in practice where protection from radiations is considered, as in the case of X-ray technicians or of workers in atomic-energy plants. There is no "safe" dose of X rays, since an individual receiving a certain number of roentgens over several years stands the same chance of having mutations

induced in his cells as an individual receiving the same number of roentgens in a few hours or minutes.

A suggestion has been made that the spontaneous mutations which arise in untreated individuals may be accounted for by exposure to the small amounts of short-wave radiations, which are omnipresent in nature. Muller, Mott-Smith, and Timoféeff-Ressovsky found, however, that the amounts of natural radiation are so small that they can account for less than 1 per cent of the spontaneous mutations that arise. The problem of the origin of spontaneous mutations thus continues to be unsolved.

Timoféeff-Ressovsky, Zimmer, and Delbrück (1935) have proposed the so-called "target theory" of induction of gene mutations by X rays, according to which a mutation is caused by a single ionization or atomic excitation within a certain "target," or a "sensitive volume." It is assumed that a single "hit" anywhere in the target will cause a gene change. The dimensions of the sensitive volume need not necessarily coincide with those of the gene that mutates, but it is interesting that computations of sensitive volumes for various genes in *Drosophila* have given dimensions of the order of magnitude of large protein molecules. The target theory is, however, not accepted by all investigators working in this field.

The mechanism of induction of mutations by ultraviolet rays is very different from that of X-ray mutations. Some ultraviolet wave lengths are much more strongly mutagenic than other wave lengths. Hollaender and Emmons, using a fungus species, and Stadler and Uber, using maize, found that the mutagenic potency of different wave lengths is roughly proportional to their absorption by desoxyribose nucleic acids, which are important chemical constituents of the chromosomes.

✓ **Induction of Mutations by Temperature and Chemical Agents.** Even before the discovery of the production of mutations by X rays, Muller had shown that more mutations occur in cultures of *Drosophila* kept at higher temperature than in those kept at lower temperature. Increasing the temperature by 10°C. more than doubles the mutation frequency. Some investigators claimed that "temperature shocks," that is, brief exposures to temperatures high or low enough to be almost lethal, increase the frequency of mutations manyfold, but more data are needed before a final conclusion can be drawn.

Attempts to induce mutation by various chemical substances were made very early in the history of mutation studies. These attempts were unsuccessful, in part because the techniques of quantitative estimation of mutation frequencies were undeveloped. Between 1930 and 1940, a number of Russian authors obtained suggestive but not quite conclusive results indicating that iodine, copper, ammonia, permanganate, mercury, and lead compounds increase mutation rates in *Drosophila* grown on nutritive

media containing these compounds. Auerbach and Robson (1946) showed that mustard gas and related substances produce increases in mutation rates in *Drosophila* which are about as substantial as those produced by heavy X-ray treatments, and this has been confirmed also in *Neurospora*. The discovery made by Demerec and his colleagues that certain cancer-inducing chemicals induce mutations in *Drosophila* has already been mentioned (p. 279). There can scarcely be any doubt that the study of chemical induction of mutations will make rapid progress in the near future.

Induction of Chromosomal Changes. Apart from mutations which are due to alterations localized at definite points in chromosomes, presumably in individual genes, X rays and other treatments induce also changes of a cruder mechanical nature, involving breakage of the chromosomes—deficiencies, duplications, translocations, and inversions (Fig. 137). Nondisjunction and loss of chromosomes are also frequent in X-rayed cells, giving rise to heteroploids.

In contrast to gene mutations, the frequency of chromosomal aberrations induced by X rays is not directly proportional to the amount of the radiation applied as measured in roentgens. It is proportional rather to the square or to the $1\frac{1}{2}$ power of that amount. In other words, doubling the amount of the treatment almost quadruples the yield of the chromosomal changes. Furthermore, Sax and other investigators working with *Tradescantia* and other plants have shown that short but intense treatments give greater yields than more prolonged but less intense ones and that continuous treatments may be more effective than interrupted ones.

One of the possible ways of interpreting these results is that X rays produce breakages in the chromosomes, the number of breakages being directly proportional to the amount of treatment. A broken end of a chromosome may rejoin with a broken end of another chromosome or another break in the same chromosome, giving rise to a chromosomal aberration (Fig. 137); or the break in the chromosome may "heal" in the old position, in which case no aberration is produced; or, finally, the broken chromosome may be lost. Furthermore, a chromosome break is capable of rejoining with another break or of healing for only a certain length of time. Translocations, inversions, deficiencies, and duplications will arise, then, only in those cells in which at least two chromosomal breaks are simultaneously available. This will occur more frequently after heavy, brief but intense, and continuous treatments than after light, prolonged, and interrupted ones. Kaufmann and Muller found, however, that in *Drosophila* the breaks do not heal but accumulate in the spermatozoa because the reunions of the broken ends of chromosomes take place after the entrance of the spermatozoon into the egg at fertilization.

Chromosomal aberrations are produced by probably all of the agents

that are known to induce gene mutations, but the ratio of the chromosomal changes to gene mutation is not necessarily the same for all these agents. Stadler has shown that, in maize, chromosomal changes are relatively more frequent after X-ray treatments, while ultraviolet radiation gives more

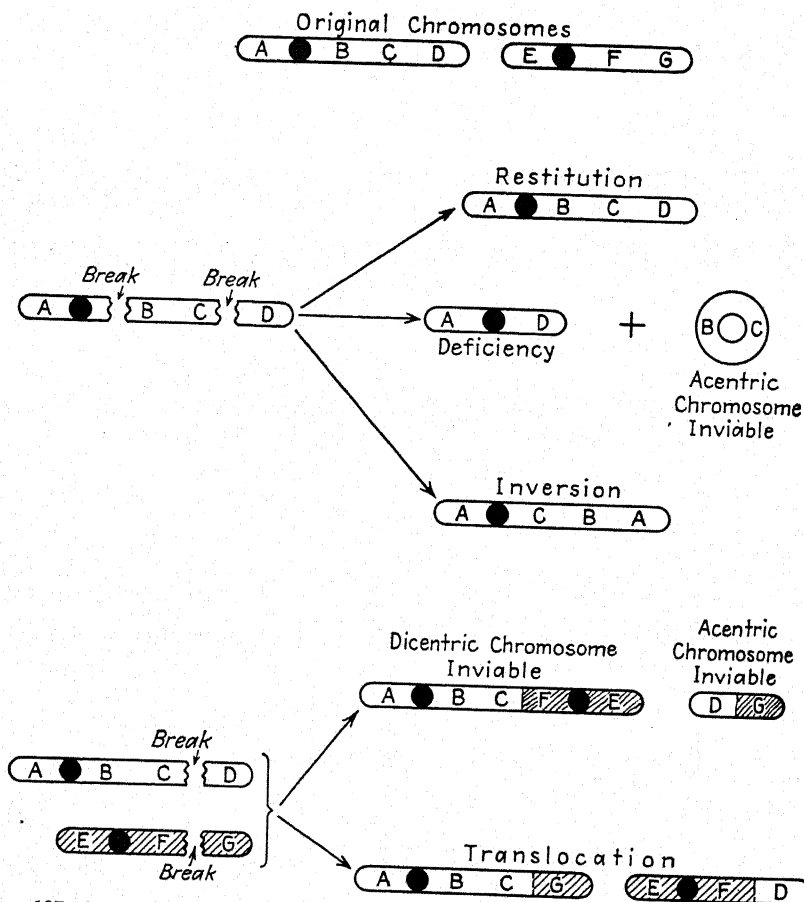


FIG. 137. Origin of chromosomal aberrations through chromosome breakage. The centromeres are shown as black circles.

gene mutations. According to Auerbach, the mutagenic properties of the mustard compounds are, in this respect, intermediate between X rays and ultraviolet.

This should not be taken, however, to mean that gene mutations can be rigorously distinguished from chromosomal aberrations. Indeed, deficiencies and duplications which are too short to be identified under the

microscope and which do not include the loci of other known genes will be inherited exactly as gene mutations are and may be mistaken for them. It is known that, in *Drosophila* and in maize, some mutations which were at first taken to represent gene mutations were later shown to be due to small chromosomal changes. Some geneticists have even contended that all mutations represent small chromosomal aberrations. Whether or not this is true, it is probable that what we call gene mutations are actually a residue left after the elimination of all changes for which a cytologically visible basis can be found. Now, how small the smallest chromosomal alterations are which can be identified under a microscope depends upon the organism concerned (since some forms have large and others small chromosomes), as well as upon the powers of observation of the investigator. The term "mutation" is used, then, in two senses. In the broader sense, it covers any change in the genotype, no matter how produced (that is, by a gene change or by a chromosomal aberration). In the narrower sense, a mutation is a change in the properties of a single gene.

Nowhere is this complexity of the mutation phenomenon more strikingly apparent than it is in *Oenothera Lamarckiana*, which by a singular accident, happened to be chosen by De Vries as the basis of his mutation theory. We have already seen (p. 275) that some of the mutants obtained by De Vries were polyploids and heteroploids. Others were not mutants at all, that is, they were not changes in any part of the genotype but were recombinations of genes which are normally carried in this plant in heterozygous condition. This is possible because, as first pointed out by Muller (1918), *Oenothera Lamarckiana* has certain properties resembling the balanced lethal heterozygotes of *Drosophila*.

Balanced Lethals. A recognition of the fact that crossing over may be suppressed (now interpreted as the effect of inversion) led to the discovery by Muller of the first case of balanced lethals. This has been of great importance, both in the practical maintenance of stocks containing lethals and in the interpretation of complex breeding behavior such as that found in *Oenothera*. The case first observed may serve as an example of the whole group. Muller studied a stock of *Drosophila* with deformed wings known as Beaded. Beaded flies mated together first produced about $\frac{2}{3}$ Beaded: $\frac{1}{3}$ normal offspring, as though Beaded were due to a single dominant gene which was lethal when homozygous. By selection he obtained from this stock one which bred practically true to Beaded but which rarely produced a normal fly. Beaded flies from this latter stock crossed with normals, however, produced 50 per cent Beaded and 50 per cent normal, showing that they were still heterozygous for Beaded. It required an extended analysis to discover how the Beaded stock which gave ratios of 2 Beaded:1 normal differed from the Beaded stock which bred true. The

first stock was found to contain in the the third chromosome a dominant mutation, Beaded, which was lethal when homozygous. Such Beaded flies were thus always heterozygous *Bd*. The true-breeding Beaded flies were also of this same composition *Bd*/+, but in addition they were found to contain another lethal *l*, also in the third chromosome and linked to Beaded. The lethal had apparently arisen by mutation during the selection experiment. In addition, the true-breeding Beaded flies contained a factor that almost completely prevented crossing over between Beaded and the new lethal, probably an inversion. Thus crosses between true-breeding Beaded flies were of the sort shown in Table XXVIII.

TABLE XXVIII

	♀ $\frac{Bd +}{+ l}$	× ♂ $\frac{Bd +}{+ l}$
F ₁ gametes (no crossing over)	$\frac{Bd +}{+ l}$	$\frac{Bd +}{+ l}$
F ₁ zygotes	$\left\{ \begin{array}{l} \frac{Bd +}{Bd +} \text{ dies, } BdBd \text{ lethal} \\ \frac{+ l}{+ l} \text{ dies, } ll \text{ lethal} \\ \frac{Bd +}{+ l} \text{ viable, Beaded} \\ \frac{+ l}{Bd +} \text{ viable, Beaded} \end{array} \right.$	

Thus nearly all flies which survived were Beaded, but a half of the total progeny died, and the Beaded stock "bred true" only in the sense that almost no non-Beaded flies survived. The rare normals which survived were due to crossing over, by which the wild-type allele of Beaded was released from its association with the lethal.

A case of balanced lethals in mice is described on page 432. In that case, the embryos, which died as a result of the two lethal mutations involved, were recovered and when studied embryologically were found to belong to the two types predicted by the theory.

The balanced lethal system obviously permits the maintenance of heterozygosity in a strain which might be mistaken for a regular homozygous line. Balanced lethal systems are widely used in laboratories to perpetuate strains of lethal, semilethal, or sterile mutants, which are difficult or impossible to maintain in homozygous strains. Such systems are also used for the detection and quantitative estimation of lethal and other mutations (see p. 321). Their bearing on the genetics of *Oenothera* is described below.

The Gene-complex Theory in *Oenothera*. *Oenothera Lamarckiana* and related forms belonging to the same genus breed essentially true, except

for throwing occasional "mutants." If, however, they are intercrossed with each other, more than a single type of hybrid appears in the F_1 generation and there is no regular Mendelian segregation in F_2 . The different types of hybrids appearing in F_1 are referred to as "twin hybrids."

The appearance of twin hybrids and other facts led Renner to the hypothesis that *Oenothera Lamarckiana* and its relatives are actually "permanent hybrids" or "permanent heterozygotes," each of which produces regularly two types of gametes. These types differ not in a single gene but in many very closely linked genes, in *gene complexes*. The set of genes carried in a given complex is constant and held together in all crosses. The various complexes, however, are so different in the gene combinations which they contain that they are distinct and rather easily recognized; to each Renner gave a name. Each of the species, according to this hypothesis, contains two of these gene complexes, clearly different; and at meiosis each separates from the other as a single entity. This analysis shows that *Lamarckiana* is composed of the gene complex called *gaudens*, containing the genes for green buds, nonpunctate stems, white nerves, broad leaves, and red flecks on the rosette leaves; and *velans*, containing genes for red-striped buds, punctate stems, narrow leaves, white nerves, and no red flecks on the rosette leaves. *Lamarckiana* may thus be described as *gaudens-velans*. Half of its pollen grains (and half of its eggs) contain the gene complex *gaudens* and the other half the gene complex *velans*.

If this is so, however, three types of offspring should arise from self-fertilization in this species: *gaudens-gaudens* ($\frac{1}{4}$), *gaudens-velans* ($\frac{1}{2}$), and *velans-velans* ($\frac{1}{4}$). Evidently the first and the last do not survive, an assumption which is supported by the fact that about half the fertilized ovules fail to produce seed, or they form seeds which are not viable. Thus only *gaudens-velans* (which is *Lamarckiana*) survives, and the species appears to breed true. The death of the two homozygous complex combinations is readily explained on the hypothesis of balanced lethal factors (p. 300) as originally suggested by Muller. One complex contains one lethal (l_1 , let us say) and the other another (l_2), and each has the dominant allele of the other's lethal. The heterozygous types L_1l_1 and L_2l_2 thus survive, but the homozygotes l_1l_1 and l_2l_2 die. ✓

It has been possible in this way to determine the genetic constitution of other species of *Oenothera*. Thus *Oenothera biennis* is *albicans-rubens*, and *O. muricata* is *rigens-curvans*. A very few species, notably *O. Hookeri*, differ from the rest in being entirely homozygous and displaying normal Mendelian breeding behavior. It should be noted that even in the heterozygous species a few genes occur which are independent of the rest of the complex in their inheritance and that these genes have been found to differ in their linkage relations in different complex combinations.

The chromosomal basis of these remarkable phenomena has been worked out by Cleland, Darlington, and others. It has been discovered that some (though not all) varieties of evening primroses have at meiosis four or more of their chromosomes attached end to end to form rings of chromosomes. Thus, *Oenothera Lamarckiana* has at meiosis a ring of 12 chromosomes and only a single bivalent (Fig. 138). Some forms have all of the 14 chromosomes present in the evening primroses united in a single ring. Now, the presence of rings of chromosomes at meiosis is characteristic of translocation heterozygotes (p. 255). If two chromosomes exchange parts as shown in Figure 115 (p. 255), a cross-shaped pairing configuration will result at the pachytene stage and a ring of four chromosomes at metaphase and anaphase of the first meiotic division (Fig. 115). If, now, another translocation occurs involving an exchange of segments between one of the chromosomes involved in the first translocation and a previously independent chromosome, the new translocation heterozygote will form at metaphase a

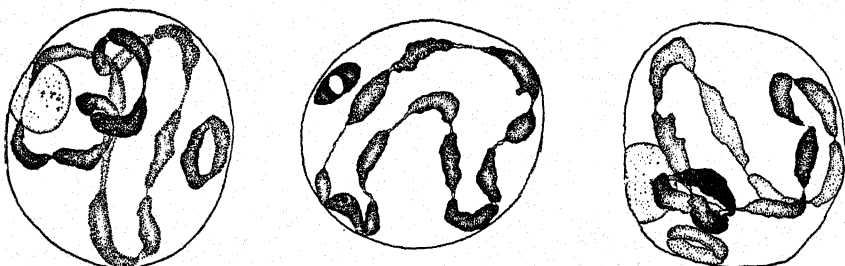


FIG. 138. Chromosomes of *Oenothera Lamarckiana* at meiosis, showing a ring of twelve and one pair. (From Cleland.)

ring of six chromosomes. Repeated translocations involving new chromosomes will eventually tie together all the chromosomes in a ring at the meiotic metaphase.

If at the meiotic division the alternate chromosomes in the ring go to the same pole and chromosomes adjacent in the ring go to the opposite pole, then the resulting cells will have sets of chromosomes containing the full complement of genes. The genes located in all the chromosomes of a set will be inherited together, exhibiting the phenomenon of apparent linkage, which, as shown on page 256, is characteristic of translocation heterozygotes. If all the chromosomes which an individual has are involved in a system of translocations which give a ring at meiosis, there will be in effect just a single linkage group of genes, as though this individual had all its genes in a single pair of chromosomes. In conjunction with the lethals, this will give the gene complexes which are actually observed in

Oenothera. In Oenothera, as in the balanced lethal systems studied by Muller (p. 300), an occasional crossover may give rise to an aberrant individual which may be mistaken for a mutant.

The genetic situation present in Oenothera is encountered very rarely outside this genus of plants, although translocations are occasionally met within populations of many organisms. A plant, *Rhoeo discolor*, has all of the 12 chromosomes tied up in a single ring, resembling those in Oenothera. The biological meaning of the singular genetic mechanism in Oenothera and *Rhoeo* is apparently the preservation of hybrid vigor (cf. p. 329) in a balanced-lethal system.

Comparison of Spontaneous and Induced Mutations. One of the outstanding characteristics of the mutation process is its seeming irregularity: mutations occur from time to time with statistically definable frequencies, but it is impossible at present to predict just what mutation, if any, will appear in a given individual at a given time. Mutations just happen. It is often found that a single individual with a single gene altered by mutation appears in the offspring of a normal parent among numerous normal sibs. Whatever cause has produced the mutational change must be highly localized in a single chromosome of a single gametic nucleus, since it did not produce similar changes in hundreds or thousands of presumably similar genes in similar nuclei in the same individual. This is the reason why Timoféeff-Ressovsky and others conjectured that gene mutations stem ultimately from intraatomic disturbances.

The mutations induced by X rays and other agents seem, in *Drosophila*, to represent the same assortment of changes that arise spontaneously. Such mutations as white eyes, yellow body, and cut wings have been observed repeatedly in untreated cultures, and they are also the commonest types of visible changes following X-ray treatments. It is justifiable to say that X rays and other mutagenic treatments so far tested merely speed up the spontaneous mutation process, rather than induce new kinds of changes. To be sure, chromosomal aberrations such as translocations and inversions arise, relative to mutations producing visible changes, more frequently in cultures treated with X rays than in untreated ones. In maize, Stadler and his collaborators found that X-ray induced mutations tend to be more extreme than spontaneous gene mutations, the induced ones representing possibly deficiencies of very small chromosome segments or even of single genes. Ultraviolet rays, however, seem to produce mutations which resemble the spontaneously arising ones more closely than the X-ray induced mutations. It can only be concluded that the mutation process is not yet under human control and that further work in this field is greatly to be desired.

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PROBLEMS

289. Most mutations are thought to be harmful rather than helpful to the organism in which they appear. Why?

290. What difference will there be in the way in which dominant mutations and recessive mutations, occurring in gametogenesis, come to expression phenotypically?

291. In studying the origin of gene mutations, is it easier to use dominant or recessive mutations? Explain.

292. From an examination of the chromosome maps of *Drosophila* do you think that mutations are equally likely to occur in all regions of the chromosome?

293. What suggestion can you make as to the reason for the high mutability of some loci as compared with others?

294. What are the relative advantages and disadvantages of the *CIB* and of the attached-X techniques of quantitative estimation of mutation frequencies in the X chromosome of *Drosophila*?

295. It has been suggested that so-called mutations are really the results of segregation from remote hybrid ancestry. Of what significance in this question is the fact that such variations are also found among the offspring of diploids which have had their origin through the (rarely occurring) self-fertilization of haploids?

296. Gametic lethals are unknown in animals, but pollen lethals are of common occurrence in plants. Explain.

297. In the *CIB* method of determining the appearance of a new lethal mutation in the sex chromosome, why is it that the presence of a new lethal in the chromosome from the irradiated male parent does not prove lethal to this female, since she already possesses one lethal in the other chromosome?

298. In the "attached-X" method how would the results be affected if the attached X's should break apart in the test female?

299. How would you determine whether a case of variegation or spotting is due to gene mutation in somatic tissue or to a gene for variegation?

300. In a balanced-lethal Beaded stock of *Drosophila* (p. 300), a few rare, exceptional flies with pink eyes were found. Pink proved to be due to a mutant gene in the third chromosome. Indicate how the following two hypotheses could be distinguished:

- (a) that a new mutation to pink has occurred;
- (b) that pink is due to recombination.

CHAPTER XII

GENES IN POPULATIONS

Up to this point we have been concerned chiefly with the behavior of genes in individuals and with the results of matings between specified individuals. In many species which have been studied in this way, the outcomes of particular crosses can be predicted in some detail. Where the genetic constitution has been well analyzed, as in *Drosophila* species or in maize, individuals and strains with desired combinations of characteristics can be deliberately synthesized. Even in man, where experimental crosses are impossible, our knowledge of genetic mechanisms is still sufficient to permit prediction in some cases. For example, if the blood groups of the husband and wife are known, the probable distribution of the blood groups of the children can be foreseen. The arrangement of genes in chromosomes and chromosomal aberrations have become known through the study of the heredity of individuals.

Problems of another type arise when we consider the operation of heredity in populations. A *Mendelian population* is a group of interbreeding individuals. As it is used in genetics, the term "population" is applicable only to those organisms which reproduce sexually and usually by cross-fertilization. Where reproduction is exclusively asexual, Mendelian segregation does not occur, and consequently clones or groups of clones are formed in which the distribution of genes cannot be established or studied by Mendelian methods. Since genes are not ordinarily exchanged between self-fertilized plants or animals, these pure lines do not constitute Mendelian populations in the sense just defined.

Thus we must ask how the genes present in the members of a Mendelian population will be transmitted and distributed in succeeding generations. For example, we may wish to know the relative frequencies of the different blood groups in the human population of New York or of Nanking, or of the United States or of China, or of the world; whether there exist certain preferred combinations of blood types or whether carriers of different blood types intermarry at random; whether the frequencies of the different types remain constant or change from generation to generation, and similar questions. The problems in which the heredity of populations rather than of individuals is considered constitute the field of *population genetics*. Since

the essence of evolution is change in the genetic constitution of populations, population genetics is also concerned with the elucidation of the mechanisms of evolution.

Genetic Equilibrium in Populations. Modern population genetics is founded on a proposition deduced in 1908 by Hardy and by Weinberg as a corollary of Mendel's principle of segregation. Suppose that a certain gene is represented in a human population by two alleles, T and t . Persons with T get a very bitter sensation from weak solutions of phenyl thiocarbamide (PTC), while to those of genotype tt it is more or less tasteless. T is a simple dominant to t . Moreover, since most persons are unaware of either their phenotype or their genotype with respect to PTC, marriages take place at random, that is, there is neither preference for nor avoidance of marriage between persons alike or different for this trait.

How frequent should such tasters and nontasters be in human populations? It may be useful at this point to warn the reader against mistakes very often made in considering this kind of problem by persons only superficially acquainted with genetics. The word "dominant" carries for many people an implication of vigor, while the word "recessive" seems to suggest weakness. This impression is not at all correct: many dominant genes in men and in other organisms cause hereditary diseases and malformations, while many recessive genes are advantageous. It is, accordingly, not at all necessary to suppose that the tasters will eventually supplant the nontasters, nor should every population have a classical Mendelian ratio of 3 tasters:1 nontaster. In reality, populations may contain *any* proportions of individuals with dominant and recessive traits. Furthermore, unless the carriers of a gene survive or reproduce at rates different from the carriers of an allele of this gene, *the relative frequencies of each gene allele in a population mating at random tend to remain constant from generation to generation.* This very important proposition represents actually a corollary of Mendel's law of segregation. It was demonstrated in 1908 and 1909 independently by Hardy in England and by Weinberg in Germany, and has come to be known as the *Hardy-Weinberg law*. This law is so important, particularly in studying human populations and in investigations concerning evolutionary phenomena, that its derivation must be thoroughly understood.

Suppose, for example, that some isolated territory, such as an island, is populated by equal numbers of homozygous tasters, TT , and of nontasters, tt , and that the members of this population intermarry at random with respect to the genes T and t . The possible marriages are, of course $TT \times TT$, $TT \times tt$, and $tt \times tt$. The initial proportions of the TT and tt individuals are 50 per cent of each, or .5 of the total. The marriages and their offspring may be represented by the following table:

TABLE XXIX

		Mothers	
Fathers		.5 TT	.5 tt
	.5 TT	.25 TT	.25 tT
	.5 tt	.25 Tt	.25 tt

The first generation will consist, therefore, of 25 per cent homozygous tasters, TT , 50 per cent heterozygous tasters, Tt , and 25 per cent nontasters, tt ; since homozygous and heterozygous tasters are phenotypically alike, the population will be 75 per cent tasters and 25 per cent nontasters. The same result is obtained more simply if we consider not marriages of individuals but simply unions of sex cells at fertilization. The original population evidently produces gametes with T and t in equal numbers, a "pool" of genes in the proportion .5 T and .5 t . If the gametes combine at random we have:

TABLE XXX

		Eggs	
Spermatozoa		.5 T	.5 t
	.5 T	.25 TT	.25 tT
	.5 t	.25 Tt	.25 tt

But what will this population be like in the next generation? Let us again consider the frequencies of gametes in the gene pool. Suppose that every individual produces equal numbers of functioning gametes. The homozygotes, TT and tt , will produce only T and t gametes, respectively; the heterozygotes, Tt , produce, according to the first law of Mendel, equal numbers of gametes with T and with t . The frequencies of the genes T and t in the gene pool will consequently be

$$T = .25 \text{ (from the homozygotes, } TT) + .25 \text{ (from the heterozygotes, } Tt) = .5$$

$$t = .25 \text{ (from the homozygotes, } tt) + .25 \text{ (from the heterozygotes, } Tt) = .5$$

Thus, the frequencies of T and t among the gametes giving rise to the second generation will be the same as in the preceding generation, and our population will consist in the first, second, and all following generations of

$$.25 \mathit{TT} \text{ (tasters)} + .50 \mathit{Tt} \text{ (tasters)} + .25 \mathit{tt} \text{ (nontasters)}$$

Now suppose that the tasters and the nontasters were not equally frequent among the original population of our territory but, for example, that the nontasters outnumbered the tasters in a ratio 3 tt :1 TT . The frequen-

cies of the alleles in the gene pool will, accordingly, be 25 per cent T and 75 per cent t , or .25 T : .75 t . The next generation will be as follows:

TABLE XXXI

		Eggs	
Spermatozoa		.25 T	.75 t
	.25 T	.0625 TT	.1875 tT
	.75 t	.1875 Tt	.5625 tt

In other words, 6.25 per cent of the individuals will be homozygous tasters, 37.50 per cent will be heterozygous tasters (or total of 43.75 per cent tasters), and 56.25 per cent will be nontasters. The gametes from which the next generation will arise will be as follows:

$$T' = .0625 \text{ (from } TT) + .1875 \text{ (from } Tt) = .25$$

$$t = .5625 \text{ (from } tt) + .1875 \text{ (from } Tt) = .75$$

The gene frequencies .25 T : .75 t and the phenotype frequencies .4375 tasters : .5625 nontasters will thus recur in every generation.

These results can be generalized as follows: Let q be the fraction of T gametes, and $1 - q$ be the fraction of t gametes in the gene pool. Then the distribution of phenotypes in the next generation will be:

TABLE XXXII

		Eggs	
Spermatozoa		$q T$	$(1 - q) t$
	$q T$	$q^2 TT$	$q(1 - q) Tt$
	$(1 - q) t$	$q(1 - q) Tt$	$(1 - q)^2 tt$

That is, $q^2 TT$: $2q(1 - q) Tt$: $(1 - q)^2 tt$.

This expression is known as the *Hardy-Weinberg formula*. Provided that we are dealing with a population (1) which is numerically so large that accidents of sampling may be ignored, (2) in which marriages are concluded at random, (3) in which the mutation from T to t or from t to T is infrequent, and (4) in which the carriers of the genotypes TT , Tt , and tt are equal in survival and reproduction rates, then the frequencies of the genes T and t among the gametes will be as follows:

$$T = q^2 \text{ [from } TT] + q(1 - q) \text{ [from } Tt] = q$$

$$t = (1 - q)^2 \text{ [from } tt] + q(1 - q) \text{ [from } Tt] = 1 - q$$

The proportions of gametes with T and t and the proportions of the zygotes TT , Tt , and tt in the population will be constant from generation to generation.

It follows that, if the frequency of the homozygous recessive class in a population is known (such as the frequency of nontasters in a human population), the frequency of the recessive gene in the gene pool may be easily calculated. The frequency of a gene is, evidently, the square root of the frequency of its homozygous phenotype. Conversely, if the frequency of a gene is known, it is easy to compute, with the aid of the Hardy-Weinberg formula, the frequency of each phenotype. For example, in the American white population, approximately 70 per cent of persons are tasters, and 30 per cent are nontasters, of PTC. The approximate frequency of the recessive gene for taste blindness is accordingly

$$t = 1 - q = \sqrt{.30} = .55$$

The dominant gene T has the frequency

$$T = q = (1 - .55) = .45$$

The frequency of homozygous tasters TT should be

$$q^2 = .45^2 = .2025, \text{ or } 20 \text{ per cent}$$

The frequency of heterozygous tasters Tt should be

$$2q(1 - q) = 2(.45 \times .55) = .4950, \text{ or } 49.5 \text{ per cent}$$

Actually the predicted ratio of $\frac{3}{4} TT : \frac{1}{4} Tt$ among all tasters has been found to hold good in the American population.

The application of the Hardy-Weinberg formula to the study of genes in populations is sometimes known as the *gene-frequency method*.

We may now state the theory of genetic equilibrium in more general terms. If a gene is represented in a panmictic population of infinite size, that is, one which is crossbreeding at random, by adaptively neutral alleles A and a in the ratio $q A : (1 - q) a$, then the frequencies of all such alleles will remain constant in the genotypic proportions $q^2 AA + 2q(1 - q) Aa + (1 - q)^2 aa = 1$ unless the frequencies are altered by

1. Mutation ($A \rightarrow a$ or $a \rightarrow A$).
2. Selection.
3. Genetic drift in small sections of the population (p. 344).
4. Differential migration

If a gene is represented in a panmictic population by several adaptively neutral alleles, A, A^B, a, \dots , the frequencies of which in the gene pool are p, q, r, \dots , then such a population at equilibrium will consist of:

$$p^2 AA + q^2 A^B A^B + r^2 aa + \dots + 2pq AA^B + pr Aa + 2qr A^B a = 1$$

In contrast to the offspring of crosses of individuals, among which different genotypes appear always in definite ratios, *e.g.*, 1:2:1, or 1:1, genes and genotypes in populations may have any frequencies. Thus, dominant genes may be rare and recessives frequent, as determined by the source and history of the population, differential migration, mutation rates, etc. No inference as to the mode of inheritance of a trait can be drawn from the frequency of this trait in a population, except that, if both homozygotes and heterozygotes are phenotypically or otherwise distinguishable and their relative frequencies agree with the equilibrium formula, then it can be concluded that the trait shows Mendelian segregation. The Hardy-Weinberg rule is, in fact, a corollary of Mendel's law of segregation.

According to the pre-Mendelian conception of heredity held by Darwin and by other early evolutionists, the genotype of a child is an alloy of those of its parents. If this view were valid, a sexually reproducing population would tend to become genotypically uniform with time. Consider, for example, a population in which some individuals are genotypically tall, others intermediate, and still others short. Suppose that matings occur at random, so that there is no tendency for tall (or for short) individuals to mate with tall, or with intermediate, or with short ones. In such a population, whenever a tall individual mates with an intermediate or with a short one, the progeny would, according to the pre-Mendelian view, be both genotypically and phenotypically intermediate; intermediate progeny would be produced also in matings of short with intermediate and tall, and in matings between intermediate individuals. After several generations, tall and short variants would disappear, and eventually the whole population would become intermediate and uniform in genotype, that is, it would become a "pure race." But Mendel's discovery had shown that sexual reproduction does not lead to disappearance of the hereditary variability present in a population. In our example, the population will always contain tall, intermediate, and short individuals, because the gene combinations that give rise to individuals of different stature are equally likely to arise in all generations. Pure races cannot be formed in sexually reproducing and cross-fertilizing organisms.

Sexual Reproduction and Variation. The relative frequencies of homozygotes and heterozygotes indicated by the Hardy-Weinberg formula will be observed only if the population is panmictic, that is, if the carriers of the genotypes AA , Aa , and aa mate at random. Not all populations are panmictic, however. Matings of individuals genotypically similar or related in descent may be more frequent than those of unrelated individuals (*homogamic* mating, or inbreeding). For example, there may be a preference for mating of tall persons with tall and of short with short (*assortative* mating). In many plants the flowers are so constructed that self-pollination is more likely to occur than cross-pollination; self-fertilization may be

even a prevailing or exclusive method of reproduction. Now, homogamic mating leads to homozygotes being more and heterozygotes less frequent than they should be at equilibrium. In self-pollinated plants most individuals may be homozygotes. Conversely, in some organisms there exists preference for mating of unlike individuals (*heterogamic* mating). This is the case, for example, in plants which are self-sterile (p. 90) or heterostylic. Heterogamy increases the frequency of heterozygotes. It must be noted, however, that neither homogamy nor heterogamy changes the gene frequencies in the gene pool, but only their combinations in hetero- and homozygotes.

In organisms that reproduce asexually, the entire progeny of an individual, known as a clone, is, barring mutation, genotypically uniform and constitutes a "pure race." Clones are especially common in microorganisms, but they also occur in some higher plants which reproduce asexually. Thus, Anderson found colonies of *Iris* (blue flags) which consisted of thousands of individuals representing a clone, and Camp observed similar clones in blueberries (*Vaccinium*).

In higher animals, in most insects, and in many plants, reproduction is exclusively sexual and cross-fertilization is obligatory. In such organisms, no two individuals are likely to carry the same genotype. Indeed, suppose that only 100 genes are represented in populations of a species by only two alleles each. This is a very conservative estimate for most species. The number of genotypes that may arise in such a species owing to sexual reproduction and Mendelian recombination is, then, 3^{100} (cf. Table VII, p. 69). Now, this number is much greater than that of all the atoms on earth. It is therefore safe to say that only a small fraction of the genotypes which a panmictic species is capable of producing are ever realized. Consequently, the genotype present in any one individual is not likely to be repeated in any other individual. Identical twins are, of course, exceptions, since they arise by asexual reproduction of a sexually produced zygote. Asexual reproduction is, accordingly, not entirely absent even in man.

In sexual and cross-fertilizing species, all or almost all individuals are heterozygotes and therefore, technically speaking, hybrids. Since the parents are always genotypically different and heterozygous, brothers and sisters always differ genotypically from each other and from both parents. In asexual and in parthenogenetic species a genotype once formed may persist for many generations and in many individuals. Sexual reproduction permits no such stability of the genotype. The germ plasm of such populations is churned by hybridization and Mendelian recombination in every generation.

Nevertheless, the gene frequencies in populations at equilibrium do not change from generation to generation.

Causes of Evolution. Since evolution consists of changes in gene frequencies, the genetic equilibrium in an evolving population must from time to time be upset. Several agencies are known which may alter the gene frequencies.

If a gene *A* changes by *mutation* to *a*, the gene pool may be losing *A* and gaining *a* in every generation. This is *mutation pressure*. Individuals that carry the allele *A* may survive more often or produce more offspring than the carriers of the gene *a*. Differential perpetuation of genes from generation to generation constitutes *selection*. In *artificial selection* the differential survival or reproduction of carriers of different genotypes is caused by human choice, while in *natural selection* differential survival or reproduction occurs independently of human will. The distinction between artificial and natural selection is sometimes not sharp. Interchange of migrants and hybridization with representatives of different populations may increase or decrease the frequencies of some genes. Finally, in populations which consist of small numbers of individuals, the gene frequencies are subject to *genetic drift*, that is, to accidental fluctuations from generation to generation.

Mutation and Viability. Since mutation is the only known method of origin of new hereditary variability, the mutation process is considered to be the prime source of the materials of evolution. This view may seem to be contradicted by the fact that most mutations that arise in any species are deleterious. A more careful consideration, however, shows the contradictions to be spurious. Mutations that are found to arise today in any species were probably arising in natural populations of that species repeatedly in the past. Now, any mutation that improves the chance of survival of its carriers in the normal environment in which the species lives has had the opportunity of becoming established as the "normal," or prevailing, condition. The normal, or "wild-type," genotype of a species incorporates the most useful mutants that have arisen in its evolution. New mutations that occur are, then, more likely to be deleterious than beneficial.

A mutation deleterious in one environment may, however, be neutral or beneficial in other environments. Thus, the mutations that make colon bacteria resistant to bacteriophage attacks (see p. 284) are neutral or even deleterious in cultures free of bacteriophages, but only these mutants survive in the presence of bacteriophages. Table XXXIII shows the viability at different temperatures of individuals homozygous for certain second chromosomes found in natural populations of *Drosophila pseudoobscura*. The chromosomes are designated arbitrarily as A, B, C, etc., and the viability is given in percentages of the average, or "normal," viability of normal flies of the same species in the same environments.

The viability of individuals homozygous for chromosomes A and B is

normal or subnormal at all temperatures. C has a superior viability at lower temperatures and is subnormal at the higher one. D is slightly below normal at $16\frac{1}{2}^{\circ}$, semilethal at 21° , and completely lethal at $25\frac{1}{2}^{\circ}\text{C}$. E is superior to normal at $16\frac{1}{2}^{\circ}$, subnormal at 21° , and semilethal at $25\frac{1}{2}^{\circ}\text{C}$. F survives best at the intermediate temperature. Not a single chromosome has been found that would make homozygotes have better than normal viability at all temperatures. In fact, every chromosome that has been found superior at some temperatures has proved inferior at other temperatures.

A mutant gene which produces deleterious effects in combinations with some genes may not be deleterious in combinations with other genes. For example, Timoféeff-Ressovsky found that the mutant bobbed (bristles)

TABLE XXXIII. VIABILITY AT DIFFERENT TEMPERATURES OF INDIVIDUALS HOMOZYGOUS FOR CERTAIN CHROMOSOMES FOUND IN NATURAL POPULATIONS OF *Drosophila pseudoobscura* (After Dobzhansky and Spassky)

Chromosomes	Temperature, C		
	$25\frac{1}{2}$	21	$16\frac{1}{2}$
A	99	98	100
B	95	89	87
C	92	109	109
D	0	43	89
E	28	73	106
F	3	39	0

in *Drosophila funebris* reduces the viability of the flies to 85 per cent of normal and that the mutant miniature (wings) reduces it to 69 per cent of normal. The combination bobbed-miniature, however, has a viability about 97 per cent normal.

The better known the properties of different genetic variants of a species, the greater the possibilities of creating environments and combinations in which each genotype might produce its optimal phenotype. Thus, the abnormal metabolism of sufferers from hereditary diabetes is brought back to normal by injections of insulin (see Chap. I). The "diabetic" genotype produces a "normal" phenotype in an environment created by insulin injections.

A *Neurospora* mutant which is unable to synthesize an essential vitamin may be successfully reared in an environment in which this vitamin is supplied; and this is true on a larger scale among crop plants, in which the discovery of the specific nutritional requirements of genetically different varieties is an important step in the successful "adaptation" of different genotypes to their optimal environments.

Nevertheless, it would be a mistake to think that every mutant genotype should be favorable in some environment. A majority of mutations are destructive in all environments. For example, one could imagine an environment in which the dominant mutation for brachydactyly (absence of the middle phalanx in fingers, making fingers short and stubby) might conceivably be useful. But such an environment is hardly imaginable for the dominant mutation for brittle bones, or for the sex-linked recessive mutation for hemophilia in man, or for the numerous lethal mutations in *Drosophila*. Mutational changes arise regardless of whether they may prove useful or not. Uncontrolled mutation would inevitably lead to decay of the genotype, since most mutations are harmful, being in the nature of hereditary diseases. A species in which mutation had ceased to occur would, therefore, gain a temporary advantage because of the nonproduction of genetically defective types. Yet in a changing environment such a species would lose in competition with more mutable rivals, which would be able to become adapted to new environmental conditions. Indeed, the mutation process supplies the raw materials by means of which biological adaptations occur in the course of evolution. Evolutionary plasticity can be maintained only if mutations take place. Deleterious mutations are eliminated, while those which are advantageous in some of the environments accessible to the species are multiplied by natural selection and eventually supplant the ancestral type.

Selection. Darwin's theory of natural selection is based on the obvious fact that only a part of the offspring of any species survive and reach the stage when they become parents of the next generation. The population of a species, however, may include many genetic variants some of which are better adapted than others to survive and to reproduce in a given environment. The better adapted variants will then constitute a relatively greater proportion of the surviving progeny than will the less well adapted ones, and the incidence of the better adapted variants will thus tend to increase. Artificial selection is a process quite analogous to the natural one, except that the variants which leave relatively greater numbers of surviving progeny are those favored by man rather than by nonhuman agencies.

The theory of selection can easily be stated in terms of genetics. Suppose that a certain gene is represented in a population by alleles A and a and that for every A allele transmitted to the next generation on the average $1 - s$ a alleles are transmitted. The value s is called the selection coefficient. Suppose, for example, that in a certain population, 99 a alleles are passed on to the offspring for every 100 A alleles. The selection coefficient is $s = .01$. The value of s may vary from zero in the case of no selection, the alleles being perpetuated at similar rates, to unity in the case in which one of the alleles is not transmitted at all.

Natural selection is not necessarily due to "survival of the fittest" in the "struggle for existence." Differential perpetuation of genes may be caused either by *differential survival* or carriers of the alleles A and a , or by *differential fecundity*, or by combinations of survival and fecundity. In diploid organisms, the heterozygotes Aa may be intermediate in survival and fecundity between the homozygotes AA and aa , or may be similar to one of the homozygotes, or superior or inferior to both. As shown above, a given gene may be beneficial in some environments and deleterious in others or favorable in combinations with some genes and harmful in other combinations. The fate of a gene allele in a population is determined by the *adaptive value* of its carriers, the adaptive value being the net result of all of the above causes. For example, carriers of the gene A may survive more frequently but may be less fecund than the carriers of a , and the adaptive values of both may be alike. Numerically, the adaptive value W , is equal to $1 - s$.

Selection against a Dominant Mutation. Selection is the pivotal agency in the evolutionary process. Furthermore, it is the most powerful instrument with which plant and animal breeders may alter the traits of cultivated plants and animals in conformity with man's needs and desires. The speed and efficiency of selection under various conditions are, accordingly, important both in theoretical and in applied biology.

Some mutant genes are dominant to the ancestral or "normal" condition, in which "normal" refers to those traits or alleles which are present in a majority of the individuals in a population or a species. Many dominant mutations have been described in man. The various forms of spotting or piebalding (Figs. 125 and 126, p. 273) which have appeared independently in different races and unrelated family lines and probably represent separate occurrences of similar mutations are nearly all dominant to the unspotted condition. A gene for night blindness is known to have been transmitted in the Nougaret family for nine generations without skipping a generation, while other dominants are responsible for brittle bones and for glaucoma, some kinds of cataracts, nystagmus, and certain other forms of genetically determined blindness. In *Drosophila* most mutants are recessive to the normal condition, but many dominants are also known (*cf.* p. 214), while in maize, although most mutations are recessive, some very striking ones such as ragged leaf and tunicate ear are dominant.

An undesirable dominant trait may be eliminated from a population if all individuals having this trait are prevented from leaving any offspring. For example, if all brachydactylous persons were sterilized or otherwise debarred from parenthood, the next generation of mankind would be free from brachydactyly, except for the new brachydactylous mutations that might arise. Dominant hereditary diseases which are sufficiently serious

to be classed as lethals (that is, conditions which prevent their carriers from reproduction although they may survive as individuals) thus destroy themselves, and their continued occurrence must be due to newly arising mutations. This is probably the case with the fatal disease epiloia, or multiple sclerosis.

A dominant disease may, however, be incompletely lethal, so that some of the carriers survive to reproductive age or produce on the average fewer children than normal individuals. This is probably true of Huntington's chorea. If a dominant trait is being opposed by sterilization or by other means, the measures may fail to prevent some of the carriers from producing offspring. This may happen, for example, because the disease sets in at an age when its carriers have already produced some children. Thus, the glaucoma mentioned above first appears in adults or even in old persons. Huntington's chorea, which is a grave disease of the nervous system caused by a dominant gene, usually appears after middle age. Some other diseases manifest themselves only in a part of the individuals which carry the genes in question, evidently because the phenotypic manifestation of these genes depends on environmental conditions. An example of this is Mongolian idiocy, which occurs more frequently in children born to mothers who have passed the age of thirty-five years than it does in children of younger mothers. A hereditary disease which is fatal to all carriers but which afflicts old people, past the reproductive age, is not counteracted at all by natural selection. A gene producing such a disease is, genetically speaking, neither lethal nor semilethal, unless it incapacitates its carriers before or during the reproductive age, at least to some extent. Hereditary predispositions to degenerative diseases of old age can be combated by genetic measures only if the carriers are identified before they reproduce.

In spite of all this, selection against a dominant trait may be quite effective. Suppose that the carriers of a dominant gene leave on the average half as many surviving children as do carriers of its recessive allele (selection coefficient $s = .5$). Then, ignoring mutation, the frequency of the dominant gene in the gene pool will be reduced by one-half in each generation. If such a gene arises by mutation, its frequency in the population will be about twice its mutation rate, because in every generation there will appear the mutants which arose in that generation, one-half of those which arose in the preceding generation, one-quarter of those which arose two generations ago, etc. Even with still less complete selection against carriers of a dominant gene ($s = .1$ or less) undesirable dominant traits in a population may be kept pretty well in check, and the same is true to a lesser extent for traits which are incompletely dominant. Deleterious dominant mutants are found very rarely in natural populations of wild species.

Selection against a Recessive Mutation. In most organisms, the muta-

tions that arise are more frequently recessive than dominant to the normal condition. Among the inheritable diseases and abnormalities that have been described in man, dominants are more numerous than recessives, but this is very likely due to the fact that the inheritance of dominants is more easily studied and thus more frequently noted in pedigrees than that of recessives. Examples of recessive mutant genes in man are albinism (Fig. 139) (absence of pigment in the skin and hair, pink iris, eyes oversensitive to light) and juvenile amaurotic idiocy (a grave nervous disturbance which appears in childhood and results in death before sexual maturity). Amaurotic idiocy may be regarded as a recessive lethal mutation.

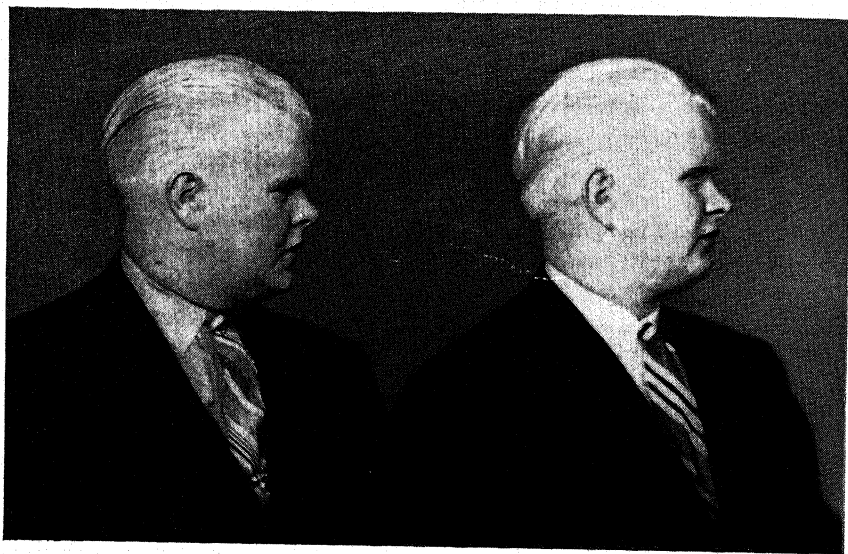


FIG. 139. Albinism, a recessive mutation, in a pair of identical twins. (From Rife, Schonfeld, and Humstead in *Journal of Heredity*.)

Suppose that a normal dominant gene A mutates to a recessive allele a . Since mutants usually appear singly among masses of unchanged representatives of a strain, the mutant individual is most likely to be a heterozygote Aa . This heterozygote will probably mate with a homozygous normal individual, $Aa \times AA$, and half of the resulting offspring will be heterozygotes Aa . A recessive mutant gene may then persist in a population for many generations always being carried in phenotypically normal individuals. Only when two heterozygotes mate, $Aa \times Aa$, will mutant individuals, aa , appear in the progeny. The probability of marriage of two heterozygotes in a panmictic population is, however, the square of their frequency. Thus, if 1 per cent of the population are heterozygotes, $.01 \times .01 = .0001$, or .01 of 1 per cent of the marriages will be between

heterozygotes, and on the average one child in four in such marriages will be homozygous for the recessive.

In the English population, about 1 person in every 20,000 is a homozygous albino. The frequency of albinos is, thus, .00005. According to the Hardy-Weinberg formula, the frequency of a homozygote in a panmictic population equals the square of the gene frequency. Therefore, $(1 - q)^2 = .00005$, and $1 - q = \sqrt{.00005} = .007$. In the gene pool of the English population the gene for albinism has a frequency of about .7 per cent and its normal allele about 99.3 per cent ($q = .993$). At equilibrium the heterozygotes in the English population will have the frequency $2q(1 - q) = 2(.007 \times .993) = .0138$, or 1.38 per cent. The heterozygotes, who are themselves normally pigmented, are thus 276 times more frequent than the homozygous albinos ($.0138/.00005 = 276$).

In general, the less frequent a recessive gene in a population, the more strongly are the homozygotes outnumbered by heterozygotes. This is very important because even if a recessive mutant gene is lethal in homozygotes (as the gene for juvenile amaurotic idiocy) it may accumulate in the population until the heterozygotes become so frequent that homozygotes are produced and eliminated at a rate sufficient to balance the inflow of the recessive genes owing to new mutation. Suppose that the recessive lethal allele a arises from A , at a rate u per generation, and that $u = .00001$ (1 mutation per 100,000 gametes). The frequency of a will, then, reach $1 - q = \sqrt{u} = \sqrt{.00001} = .0032$, or .32 per cent of the gene pool, at which frequency the equilibrium between mutation and elimination will be established.

Since heterozygous carriers may be more numerous than recessive homozygotes, selection against a recessive trait is less effective than that against a dominant trait. Suppose that recessive homozygotes are destroyed or sterilized generation after generation ($s = 1.00$, complete selection). If the frequency of the recessive gene in the gene pool of a population is initially $1 - q = .5$, the population will consist, according to the Hardy-Weinberg formula, of 25 per cent AA , 25 per cent aa , and 50 per cent Aa . The heterozygotes are, then, twice as numerous as are homozygotes (Table XXXIV). If all recessive homozygotes are prevented from reproduction, the recessive genes carried in the heterozygotes are still passed on to the next generation. As shown in Table XXXIV, the frequency of the recessive gene in the gene pool of the next generation will be .33, and the frequency of recessive homozygotes will have dropped from 25 per cent to 11.11 per cent.

This is good progress. But notice that the heterozygotes are now already 4 times as frequent as the homozygous recessives. After 8 more generations of total selection against the recessive trait, the gene frequency drops

to $1 - q = .1$, and the frequency of recessive homozygotes to just 1 per cent of the population, the heterozygotes being 18 times more frequent (Table XXXIV). To depress the frequency of recessive homozygotes to .1 per cent, 21 more generations of selection are needed; to cut it to .01 per cent (twice the frequency of albinism in the English population), 70 more generations are needed.

TABLE XXXIV. EFFECTS OF COMPLETE SELECTION AGAINST A RECESSIVE TRAIT

Generations	Gene frequency ($1 - q$)	Recessive homozygotes, %	Heterozygotes, %	Dominant homozygotes, %
1	.500	25.00	50.00	25.00
2	.333	11.11	44.44	44.44
3	.250	6.25	37.50	56.25
4	.200	4.00	32.00	64.00
5	.167	2.78	27.78	69.44
9	.100	1.00	18.00	81.00
10	.091	.83	16.53	82.64
20	.048	.23	9.07	90.70
30	.032	.10	6.24	93.65
40	.024	.06	4.76	95.18
50	.020	.04	3.84	96.12
100	.010	.01	1.96	98.03

The selection against a recessive trait may, however, be only partially effective, because the gene is not lethal when homozygous (in natural selection), or because only some of the recessive homozygotes show the phenotypic trait by which they may be identified, or finally because some homozygous individuals escape sterilization. Table XXXV shows the effects of

TABLE XXXV. EFFECTS OF PARTIAL SELECTION AGAINST A RECESSIVE TRAIT ON THE FREQUENCY IN PER CENT OF INDIVIDUALS HOMOZYGOUS FOR THE RECESSIVE GENE

Generations	$s = 1.0$	$s = .50$	$s = .10$	$s = .01$
1	1.00	1.00	1.00	1.00
10	.25	.46	.84	.98
20	.11	.26	.71	.97

various degrees of selection in a population which initially contains 1 per cent of homozygous recessive individuals (that is, the initial gene frequency being .1). With complete selection ($s = 1.0$), 10 generations are needed to cut the frequency to one-quarter of its former value and 20 generations

to reduce it to .11 per cent. If the gene is semilethal, or if selection misses half of the homozygotes ($s = .5$), 20 generations are needed to reduce the frequency of homozygous recessives to about one-quarter of its former value. With selection $s = .01$, 20 generations bring a reduction only from 1 per cent to .97 per cent. Since many hereditary diseases and defects in man are due to recessive genes each of which, taken separately, is rare (although they may be very frequent in the aggregate), freeing the population from these afflictions by means of sterilization or similar measures would be a very slow process.

Concealed Hereditary Variability in Populations. Most species contain some individuals of distinct phenotypes, and these differences are frequently hereditary. Considerations presented in the foregoing paragraphs suggest, however, that apart from this phenotypically apparent variability there may exist also a concealed variability, due to the presence in populations of recessive mutant heterozygotes. This simple idea, first clearly stated by Chetverikov in 1926, proved to be very fruitful.

Mutants frequently mentioned in foregoing chapters, such as vestigial wings, black body color, white eyes, and others, occur but very rarely in populations of *Drosophila* living in their natural habitats. But these mutants, so useful in genetic experiments, are nevertheless not purely laboratory products, since these and many others, including numerous recessive lethal and semilethal genes, do occur in natural populations in heterozygous individuals. In order to study the genetic variability concealed in heterozygotes, samples of natural populations of *Drosophila* must be taken and submitted to a laboratory analysis. This analysis involves a series of crosses resembling in principle the *CIB* method of detection of sex-linked mutants (see p. 280), except that, when autosomal recessives are to be detected, individuals are obtained which are homozygous for certain chromosomes derived from wild flies. Any recessive genes that may be contained in these chromosomes manifest themselves in the phenotype of the homozygotes. Other chromosomes, carrying "marking" mutant genes of laboratory origin, are used to enable the investigator to recognize the flies homozygous for the wild chromosomes.

Samples of natural populations of *Drosophila pseudoobscura* analyzed by the above method proved to be replete with recessive mutant genes carried in heterozygotes. Table XXXVI shows the percentages of second, third, and fourth chromosomes of wild flies which contain recessive mutants of various kinds. X chromosomes contain few mutants.

It can be seen in Table XXXVI that, respectively, 21, 14, and 25 per cent of the second, third, and fourth chromosomes found in natural populations are lethal or semilethal in homozygotes. Since every fly carries two second, two third, and two fourth chromosomes, the proportion of the flies in the population which contain one or more lethals or semilethals in hetero-

zygous condition is very high. With the aid of the Hardy-Weinberg formula, it can be computed that only about 25 per cent of the flies are free of lethals and semilethals, while about 75 per cent carry one or more of these recessive mutant genes. Flies with two or more lethals are normally viable if the lethals are not allelic. The numbers of kinds of genes which produce lethal alleles by mutation are quite large, at least 289 in the third chromosome of *Drosophila pseudoobscura* according to Wright.

Table XXXVI shows also that, among the chromosomes which contain neither lethals nor semilethals, many chromosomes contain deleterious recessive viability modifiers. A deleterious viability modifier is a gene (or a gene complex) which reduces the viability of its carriers to an extent smaller than a semilethal genes does (that is, kills less than a half of the

TABLE XXXVI. FREQUENCIES IN PER CENT OF CHROMOSOMES WITH RECESSIVE MUTANTS IN NATURAL POPULATIONS OF *Drosophila pseudoobscura* (According to Dobzhansky, Holz, and Spassky)

Effects in homozygotes	Chromosomes		
	II	III	IV
Lethals and semilethals.....	21	14	25
Viability modifiers, deleterious.....	21	30	41
Viability modifiers, favorable.....	1	.5	.5
Sterility genes.....	13	Unknown	8
Visible structural changes.....	4	3	2

homozygous individuals). It has been computed that only about 11 per cent of the wild flies do not carry at least one chromosome with deleterious modifiers. Furthermore, 8 to 13 per cent of the chromosomes carry recessive genes that make homozygotes sterile, and 2 to 4 per cent carry genes that produce externally visible changes in the structure of the fly's body. Finally, less than 1 per cent of the chromosomes contain favorable viability modifiers, which "improve" the viability of the homozygotes to a level above the average normal for wild flies. These modifiers have been discussed above (see p. 314).

Dubinín and his colleagues, Timoféeff-Ressovsky, Spencer, and others have found that species of *Drosophila* other than *Drosophila pseudoobscura*, such as *D. melanogaster*, *D. funebris*, *D. immigrans*, and others, also carry great numbers of recessive mutant genes concealed in phenotypically normal heterozygotes. Percentages of individuals in some varieties of maize that are heterozygous for various recessive mutant genes are shown in Table XXXVII. These data have been obtained by artificially self-pollinating individual plants of different varieties of commercial field corn.

If an individual is heterozygous for the recessive gene producing, for example, white seedlings, about one-fourth of the seedlings in the progeny are white. Some of the genes found (white and yellow seedlings, defective endosperm, and germless and viviparous seeds) are lethal when homozygous, while others (virescent, pale green, and dwarf seedlings) may be classified as semilethals.

Although no exact quantitative data on the incidence of deleterious recessive genes are available for human populations and for populations of other organisms, it is reasonably certain that the commonness of concealed

TABLE XXXVII. PERCENTAGES OF INDIVIDUALS HETEROZYGOUS FOR VARIOUS RECESSIVE DEFECTS ENCOUNTERED IN SOME VARIETIES OF MAIZE
(Data of M. M. Rhoades)

Variety	White seedlings	Virescent seedlings	Yellow seedlings	Glossy seedlings	Pale green seedlings	Dwarf seedlings	Striped seedlings	Defective endosperm	Liguleless	Miscellaneous
Hays Golden....	12	46	3	3	5	2	3	23	1	3
Reid Yellow										
Dent	14	66	9	1	2	1	5	2
Pride of Saline..	28	39	6	2	2	2	11	9
Golden Glory....	15	40	1	4	17	1	20	3
Woodburn Dent.	4	34	4	2	8	10	34
Midland Yellow										
Dent.....	5	62	2	3	2	9	10
Silver King.....	11	64	3	2	5	11	5

harmful genes found in *Drosophila* and in maize is a general phenomenon in cross-fertilizing animals and plants. Evidence of this is provided by observations on inbreeding and on heterosis, or hybrid vigor.

GENES IN INBRED POPULATIONS

Inbreeding. Breeders of domestic animals and plants have known for centuries that inbreeding, or mating of individuals closely related in descent, often results in lessened vigor, reduced size, and diminished fecundity and ultimately in inability of the stock to survive. The fact that in many of the higher plants self-pollination is prevented or rendered relatively infrequent by various structural or functional means and similarly that in most animals the progeny arise from matings between different individuals convinced many earlier biologists that self-fertilization was an unnatural and thus presumably harmful process. The extensive experiments of Darwin with plants seemed to lead to the same conclusion, since in most species the

offspring arising from self-fertilization were clearly less vigorous than those from cross-fertilization. It was his opinion, shared by most biologists until recent years, that *the process of inbreeding itself* in some way produced these injurious effects.

Objections to such a general conclusion, however, began to appear when the problem was reinvestigated by carefully controlled experiments and in the light of a knowledge of Mendelian inheritance. Attention was called to the fact that many species of plants, such as peas, beans, barley and oats, are normally self-fertilized but nevertheless compare favorably with cross-fertilized species in vigor, fecundity, fertility and viability. Furthermore, several animals with which careful inbreeding experiments have been carried out do not invariably show the expected decrease in size and vigor. Thus, in the strain of albino rats studied by King, brothers were mated to sisters for 25 generations, at the end of which period the animals

TABLE XXXVIII. THE EFFECT OF CONTINUED SELF-FERTILIZATION UPON THE HEIGHT OF FOUR LINES OF DENT CORN (*Data from Jones*)

No. of generations selfed	A, height, inches	B, height, inches	C, height, inches	D, height, inches
0	117	117	117	117
1-5	87	81	91	77
6-10	97	84	88	82
11-15	97	84	87	83
16-20	88	85	*	75
21-25	81	75	71
26-30	92	80		

* Lost.

compared favorably with crossbred stock used as controls. It should be noted, however, that in the inbred race only the most vigorous animals were selected for breeding. In *Drosophila* inbreeding results in rapid loss of vigor and fecundity in some strains, while other inbred strains are not appreciably below normal cross-fertilized ones in vigor. In man, some marriages between relatives give progeny afflicted with hereditary diseases, while other such marriages produce healthy offspring, as witnessed by the maintenance of ancient Egyptian families for many generations by brother-sister matings. It is evident that inbreeding is neither invariably nor uniformly deleterious.

Most extensive studies on inbreeding have been carried out in maize. The work was begun independently by Shull and East in 1905 and developed by Jones, Sprague, Jenkins, and many others. Maize is normally cross-pollinated but can readily be self-pollinated experimentally. The

first generation coming from self-pollination of a normal crossbred variety is quite generally inferior to the parental generation in vigor and size of its plants, as well as in grain yield. If self-pollination is continued, the deterioration continues for several generations at a progressively decreasing rate. Unless the inbred line becomes extinct, as it frequently does, a point is reached after which further inbreeding has no effect (Table XXXVIII).

Inbreeding experiments involving 23 generations of brother-sister mating in guinea pigs have been carried out by Sewall Wright. The effect of inbreeding was to produce a decline in size and other elements of vigor. The mortality at birth and between birth and weaning, body weight, growth, size and number of litters, and resistance to tuberculosis were adversely affected, and the inbred animals were inferior to outbred controls in these respects. Just as in maize, there was here observed a conspicuous differentiation among the various inbred families, with fixation of various traits in each.

Heterosis. Hybrid vigor, or *heterosis*, is the converse of the deterioration which follows inbreeding. Hybridization of weakened inbred strains results in an immediate restoration of vigor, size, and fertility in the F_1 hybrids.

Plants show the phenomenon of heterosis particularly well. Thus in maize the F_1 hybrids between inbred homozygous strains show a marked increase over the parent strains in height, frequently show a gain of 150 to 200 per cent in yield, and have thicker stalks and larger leaves, ears, and seeds than have their inbred parents. This immediately restores all the size lost by inbreeding, and the F_1 plants are frequently as large as the original heterozygous stocks. They further show a striking uniformity in physical traits as compared with normal crossbred stocks, a result evidently due to the homozygosity which their parent lines had attained. A remarkable feature of this vigor, which reaches its maximum in the first hybrid generation, is that it cannot be fixed but rapidly disappears again under inbreeding (Fig. 140).

Practical utilization of the hybrid vigor in maize has given splendid results thanks to the development of the so-called "hybrid corn" in the United States. Numerous inbred lines have been isolated from the cross-pollinated field varieties. These inbred lines themselves are always inferior to the varieties from which they have been derived, but some inbred lines produce remarkably vigorous and high-yielding hybrids when intercrossed. The fields are then planted with hybrid seeds produced on special seed farms. The yield obtained from hybrid seeds is at least 20 per cent higher (and often more) than that given by the original crossbred varieties.

Heterosis also occurs in animals. Crossbred animals and "mongrels," although of little value for breeding, may be superior to purebred animals

in vigor and other bodily characters. Use is often made of this fact in breeding them for market. In only a few animals, however, have controlled experiments been carried out. In *Drosophila* much evidence has been obtained which indicates that size and fecundity increase when strains are crossed, particularly if they have been inbred previously. The same results have been found in guinea pigs, fowls, and other animals (Fig. 141).



FIG. 140. Heterosis in maize followed by decline in height after inbreeding. Representative plants of the two parent strains at left, followed by average plants from the F_1 , F_2 , ... F_8 generations of inbreeding toward the left. (From Jones.)

Genetic Interpretation of Heterosis. The genetic theory of heterosis was suggested by Shull and developed especially by Jones and East. It starts from the assumption, vindicated by the recent findings in *Drosophila* and maize discussed on pages 322 to 323, that populations of cross-fertilizing species consist of individuals many of which carry deleterious recessive genes in heterozygous condition, concealed by their normal dominant alleles. Now, if such individuals are inbred, types homozygous for the deleterious recessives will appear in the offspring, leading to deterioration of vigor. Different inbred lines deteriorate, however, because of homozygosis for *different* recessive genes or gene complexes. Intercrossing of inbred lines leads to formation of hybrids in which the deleterious recessives contributed by one parent are covered up by their dominant alleles contributed by the other parent. Hence, the heterozygous state is restored, and hybrid vigor, or heterosis, is the result.

If this hypothesis is correct, it should be possible to obtain individuals

homozygous for all of the dominant factors present in the hybrid and thus make hybrid vigor permanent in a true-breeding race; but in actual practice this has been found very difficult or impossible to do. Jones has reconciled this result with the hypothesis, however, by assuming that there are a relatively large number of factors affecting size and vigor and that each chromosome ordinarily may contain several of them. In inheritance they must therefore occur in groups of linked genes. A single group would be expected to contain some dominant alleles for increased size and some recessive ones for lesser growth. The hybrid would possess the dominant member of each pair, but the recombination of all of these

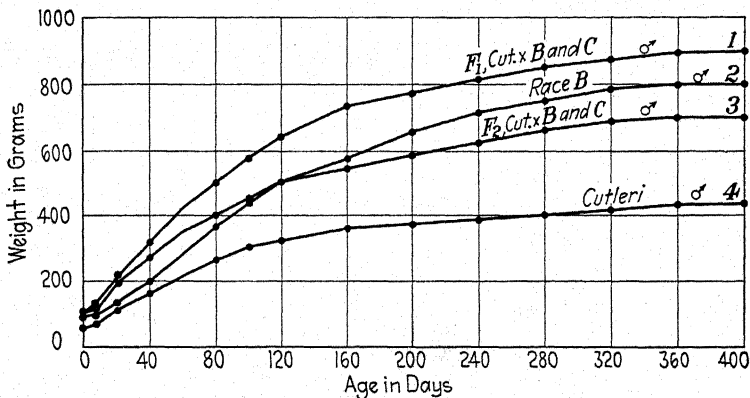


FIG. 141. Heterosis in guinea pigs. Growth curves of males of a large domesticated race of guinea pigs (race B, line 2); of a small wild race from Peru (*Cavia cutleri*, line 4); and of the F_1 (line 1) and F_2 (line 3) males from a cross of race B (and race C of similar size) with *Cavia cutleri*. The F_1 animals exceeded both parent races in growth rate and final size. The F_2 animals were distinctly smaller. (After Castle.)

together in one gamete would be extremely rare, since the genes do not assort independently as they would if there were but one to a chromosome. Their genetic behavior is necessarily complicated by their linkage relations, and a series of rarely occurring crossovers of a very precise sort would be necessary to obtain all the dominant genes in the same gamete.

Heterosis in Different Organisms. We have seen that inbreeding is regularly deleterious in some organisms (maize), occasionally deleterious in others (*Drosophila melanogaster*), and not deleterious at all in still others (wheat). Such differences are conditioned by the reproductive biology of the respective species. Where self-fertilization is, as in wheat, the normal method of reproduction, recessive deleterious mutants become homozygous very soon after their origin and are eliminated almost as rapidly and efficiently as are deleterious dominant mutations. In wheat, homozygosis for most genes is the normal situation to which the

species is adapted in the course of its evolutionary development. Accordingly, inbreeding in wheat leads to no diminution of vigor, and outbreeding brings, as a rule, no heterosis.

In species that reproduce by cross-fertilization, deleterious dominant mutants are promptly eliminated, while, as we have seen, deleterious recessive mutants are allowed to accumulate in heterozygotes. Consequently, most individuals in natural populations of *Drosophila* are heterozygotes for deleterious recessives in the autosomes. Few if any deleterious genes are found in the X chromosomes in *Drosophila*, because recessive sex-linked genes manifest themselves in the phenotype of males and deleterious mutants are eliminated. Thus, inbreeding leads to degeneration in most crossbred strains. But if, by chance, the parents of an inbred line happen to be free of concealed deleterious recessives, no loss of vigor following inbreeding is observed. The same situation obtains, probably, in populations of domesticated animals, and possibly in populations of the human species as well.

If every chromosome of every individual of a species carries several deleterious recessive genes, inbreeding will invariably lead to degeneration and outbreeding to spectacular heterosis. It is possible that this is the situation in maize. An alternative possibility is that heterosis may also be produced in individuals heterozygous for two alleles, such as A^1A^2 , while each allele may be deleterious in homozygous condition (A^1A^1 and A^2A^2 being inferior in vigor). Such a situation is especially likely to arise in cross-fertilizing species which normally exist in very large populations, in which inbreeding would normally be rare. The heterozygous and heterotic condition will, then, be the state to which the species is adjusted in the process of evolution. In such species, inbreeding constitutes a breakdown of the mode of reproduction to which the species is adapted, and intercrossing of inbred lines restores the normal reproductive biology. This interpretation is supported especially by the data on the heterosis observed in inversion heterozygotes in *Drosophila pseudoobscura* (see p. 340).

Permanent Heterozygotes, Parthenogenesis, Apogamy, and Asexual Reproduction. Assume that the heterozygous condition, A^1A^2 , is superior in vigor to the homozygous status, A^1A^1 and A^2A^2 . Matings of two heterozygotes A^1A^2 will, however, produce offspring consisting of about 25 per cent A^1A^1 , 50 per cent A^1A^2 , and 25 per cent A^2A^2 . Thus, half of the zygotes belong to the ill-adapted homozygous types. A genetic mechanism that would avoid the production of the ill-adapted types would, naturally, prove advantageous in evolution. This consideration makes understandable some aberrant genetic situations which are known in certain organisms.

Permanent heterozygotes in evening primroses, *Oenothera*, have already been discussed in Chapter XI (p. 301). In *Oenothera*, an individual usually forms only two kinds of gametes carrying different complexes of genes, the complexes being held together by peculiar systems of translocations. Only zygotes heterozygous for the two complexes survive, homozygotes being eliminated or not produced at all. The latter condition obtains if one of the complexes causes abortion of the pollen grains and the other, abortion of the ovules. This singular genetic mechanism makes heterosis compatible with self-fertilization, although normally self-fertilization is the strictest form of inbreeding and self-fertilizing plants are usually homozygous. Many varieties of *Oenothera* have flowers adapted for automatic self-pollination.

In some species belonging to diverse groups of organisms, the processes of fertilization and meiosis, which constitute the essence of sexual reproduction, degenerate or are lost entirely. This retrogression of sexuality may occur in a variety of ways, the genetic consequences of which are quite different. Thus, meiosis may take place, but the egg cells may develop without fertilization (*parthenogenesis*). Individuals arising by parthenogenesis may be either haploid as in bees, wasps, ants, and their relatives (see Chap. XV) or diploid as in various insects and worms (in which the second meiotic division is abortive or chromosome doubling occurs without nuclear division). In either case, parthenogenetically produced offspring will be largely or completely homozygous. However, meiosis, with its chromosome pairing and disjunction, may be suppressed, and diploid cells may give rise without fertilization to individuals of the next generation. This is called *apogamy*. It occurs in many species of plants and in a few animals. Finally, *asexual reproduction* by fission (buds, bulbs, tubers, or runners) dispenses altogether with specialized sex cells and sex organs. Offspring produced apogamically or asexually carry the same genes which were present in the parent unless mutation occurs. If the parent was a heterozygote, so is the offspring.

In oranges, lemons, and other citrus species, pollination of the flowers stimulates the development of seeds and fruits, but the seeds are produced mostly by apogamy. Individual citrus plants are highly heterozygous. Fertilization occasionally takes place, but the plants that grow from sexually produced seeds often differ greatly from the parental trees, because of the occurrence of complex gene segregations. Similar high heterozygosity is characteristic of most cultivated apples. Accordingly, apples are propagated in practice almost entirely asexually, by grafting, rather than by being grown from seeds. Among the varieties of the genus *Rubus*, such as blackberries, raspberries, and related types, many apogamic forms are known, although some sexual species also exist. The cultivated types

of these plants are propagated asexually. Apogamy is also common in some grasses. Strains of the bluegrass, *Poa alpina*, may carry 26, 31, 33, 35, 37, 38, and higher numbers of chromosomes in their somatic cells, and the apogamically produced progenies retain the parental chromosome numbers. This variation of chromosome numbers arose doubtless by polyploidy and aneuploidy through the occurrence of trisomics and monosomics (cf. p. 246). Aneuploids do not breed true when reproducing sexually, but they can be perpetuated by apogamy. Strains of *Poa alpina* and related forms can occasionally be crossed, and the sexually produced F_1 plants are highly variable on account of segregation. But the F_2 progenies obtained apogamically from the F_1 segregates show no further segregation, thus inverting the usual behavior of hybrids.

Relatively few organisms reproduce exclusively parthenogenetically, apogamically, or asexually. More often these aberrant methods of reproduction alternate at more or less regular intervals with the usual sexual one. Such alternation permits the species to exploit the advantages both of sexuality and of asexual reproduction. Sexual reproduction engenders numerous gene or chromosome combinations, some of which prove advantageous to the organism. These advantageous combinations are then perpetuated by asexual reproduction for a time in a state that protects them from being shattered by more recombination.

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PROBLEMS

301. The frequency of juvenile amaurotic idiots at birth in Sweden is .000038. By means of the Hardy equilibrium formula, calculate the probable frequency of Swedish persons who are heterozygous for this gene.

302. The frequency of three blood types in a sample of 300 persons is as follows: Type M 42.7 per cent; Type MN 46.7 per cent; Type N 10.7 per cent. Does this fit the assumptions of segregation and random mating? From the above, give the probable genotypes of the three blood types.

303. In an isolated population of poultry which has gone wild and is mating at random, three color types are found as follows: black 490, gray 420, white 90. Suggest a hypothesis to account for these proportions.

304. Several investigators have each found that about 70 per cent of Americans get a bitter taste from the drug phenyl thiocarbamide (PTC) and 30 per cent get no bitter taste from it, that is, they are "taste blind" to PTC. Assume that taste blindness is recessive to normal. What proportion of marriages between normal and taste-blind persons have no chance of producing a taste-blind child?

305. Snyder found 278 taste-blind, tt , offspring out of 761 tested offspring from marriages of taster by taste-blind. From the gene frequencies of t on page 310, calculate the expected frequencies of T and t children from the matings above, and test the goodness of fit of the expected to the actual frequencies. What conclusions can be drawn?

306. In the tenth generation of the offspring of a self-fertilized plant heterozygous for Aa , what proportion would there be of heterozygous individuals?

307. Why should 6 generations of self-fertilization be as effective in reducing heterozygosity as 17 generations of brother-sister mating?

308. To obtain maize seed which will produce plants having the large size and yield and other advantages of heterosis, it is impractical to use the seed from crossed inbred lines since the yield is relatively small. What means can you suggest for obtaining a high yield of seed which will produce heterotic plants?

309. Compare the chances for establishment of a new viable mutation in a self-fertilized and in a cross-fertilized population.

310. Using the tables and directions in the paper of Bonnier (1947) (*cf.* References) work out the consequences of mating in each generation between a single brother-sister pair in a population in which the alleles A and a are initially equally frequent.

311. In a population mating at random (as in man) what will be the relationship between effective size of the interbreeding population and the frequency of cousin marriage?

312. What is the chance that a child of a marriage between first cousins will be homozygous for a gene for which only one of the common grandparents was heterozygous, Aa while all other parents were AA ?

313. The proportion of recessives aa in a large crossbreeding population in equilibrium is .04. Assuming aa to be normal in viability and fertility, what should be the proportion of Aa in the population? If (as in man) matings could not be controlled, how could the theoretical and actual proportions of Aa be compared?

CHAPTER XIII

GENETICS OF RACE FORMATION

The observations of students of genetics over the last fifty years make it very probable that mutational changes in genes and chromosomes occur in all organisms. The mutation process thus supplies the raw materials from which, and only from which, evolutionary changes can arise. The great majority of newly occurring mutations are, to be sure, deleterious in combination with the normal genotype and in the normal environment of the species, and most of them are thus eliminated by natural selection. Dominant mutations are retained in populations only if they happen to be useful or neutral in survival and reproduction; deleterious dominant mutations are usually lost very soon after they arise. The situation is different with recessive mutants. In species which reproduce sexually and by cross-fertilization, recessive mutants accumulate in heterozygous condition, because even a lethal recessive mutant is sheltered from natural selection by its normal dominant allele. Therefore, cross-fertilizing populations carry a great deal of concealed hereditary variability. Mutants that are deleterious in some environments may, however, be useful in other environments, and mutants that are deleterious when taken separately may be advantageous in combinations. Owing to natural causes as well as to man's activities, the environment in which a species lives may change. Some of the mutants which were neutral or deleterious in a former environment may prove useful in a changed one. Such mutants will increase in frequency and eventually become "normal" in populations exposed to the changed environment. These populations will, then, form a new race, which may later develop into a new species. The purpose of the present chapter is to review the evidence bearing on the theory of race formation.

Adaptive Changes in Experiments. Adaptive changes can be observed directly in organisms which multiply rapidly and which can be bred under observation in large numbers. These requirements are most easily satisfied in microorganisms; hence the emergence of strains of viruses, bacteria, and lower fungi adapted to various culture conditions has been known in microbiology for some time. Examples of the formation of strains of bacteria resistant to bacteriophages and to penicillin have been discussed previously (pp. 284-287). Adaptive changes can, however, be observed in more complex organisms as well.

We have seen (p. 322) that in *Drosophila pseudoobscura* and other species a large proportion of chromosomes in wild flies contain recessive mutants that act as lethals, semilethals, or deleterious modifiers of viability when homozygous. Seven strains homozygous for such deleterious genes were chosen and bred for 50 consecutive generations in laboratory cultures. By placing some four dozen parents in each culture, the cultures were deliberately overcrowded with larvae, which accordingly developed under conditions of severe competition. In addition, each of the seven strains

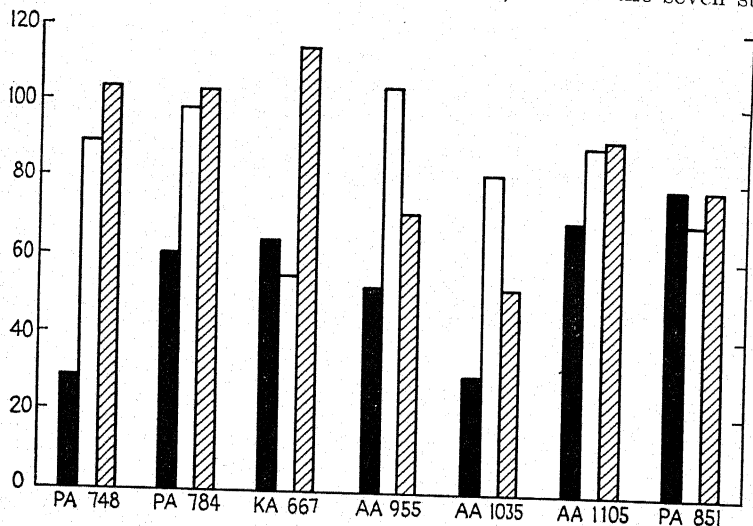


Fig. 142. Results of fifty generations of natural selection for viability in seven strains of *Drosophila pseudoobscura*. Ordinates, per cent of normal viability; black, viability of the strain at beginning of the experiment; white, viability after 50 generations, untreated; cross-hatched, viability after 50 generations of exposure to low doses of X radiation. (From Dobzhansky and Spassky.)

was subdivided into two parallel lines; in one line the parents were treated with X rays in every generation, and in the other lines the parents were left untreated. It was expected that any mutants that might appear with favorable effects on the viability of the flies or their larvae would be multiplied by natural selection and could become established in the strains. Since the strains had to begin with a low initial viability on account of the abnormal genetic constitution (homozygous for deleterious recessive mutants), it seemed reasonable that mutants might arise bringing the viability back to normal.

The outcome of the experiment is shown in Fig. 142. In this diagram, the height of the black columns shows the initial viability of the seven strains expressed in percentages of the average viability of normal flies of the same species. The white columns show the viability of the same

strains after 50 generations of the strongest selection; and the hatched columns show the viability of the same strains after 50 generations of treatment with X rays combined with selection. It can be seen that six out of the seven X-rayed and five out of the seven untreated strains showed substantial, and in some cases striking, improvements in survival ability. Now, there can be no reasonable doubt that many more deleterious than beneficial mutations had arisen in these strains, particularly in the X-rayed lines. The deleterious mutants had, however, been eliminated, and the beneficial ones increased and established by the natural selection which took place during the 50 generations of breeding. Evolutionary changes leading to improved adaptation of some strains to their environments have, thus, been proved to take place.

Artificial Selection. As pointed out by Darwin, the varieties of domesticated animals and plants now in existence have been developed from their wild ancestors by selection consciously or unconsciously applied by man. It is enough to compare, for example, wild boars with modern specialized breeds of pigs or a wild apple tree with one of an improved variety to appreciate how great may be the changes induced by selection in time intervals which, speaking geologically, are very short. In fact, in some cases there is still no agreement among authorities as to just which wild species gave rise to the modern domesticated ones, so extensive have been the changes under domestication.

The simplest technique used by breeders is *mass selection*, which consists in systematically picking the "best" individuals to become parents of the next generation. The "best" are, of course, individuals which show the features most desired by the breeder, such as large size, high yield of grain, milk, or the like, or high speed on the race track. In a sexually reproducing species, in which a large variety of mutant alleles have been accumulated, mass selection may continue to be very effective for many generations. Indeed, Mendelian recombination of genes available in the population with which selection is started may potentially be able to produce so great a variety of genotypes that many generations of selection will be necessary to exhaust the possibilities of improvement of the breed by this means, even disregarding the new mutations that may arise during that time.

The rate of progress of breeding work will also depend upon the ability of a breeder to pick for reproduction individuals that are not only phenotypically but also genotypically superior to other members of the population. For example, the milk yield in dairy cattle depends upon the genotype of the animals as well as upon their nutrition and general health. A high-yielding cow may carry a less favorable genotype than another cow which gives lower yields. The only possible way to recognize the geno-

typic superiority, apart from as careful equalization of the environmental conditions as may be obtained, is a *progeny test*. That is, if the progeny of a given cow has produced, other conditions being equal, a higher average milk yield than the progeny of another cow, the former probably carried the superior combination of genes. In the case of bulls the progeny test is obviously the only criterion available.

In plants such as wheat, which reproduce mainly by self-fertilization, a field population is an aggregate of more or less homozygous pure lines, which exchange genes only seldom by occasional crossing. The *pure-line method* of breeding consists in isolating those pure lines which possess the most desirable set of characteristics. As shown by Johannsen, selection is not effective in homozygous pure lines, because selection merely chooses among the existing variety of genotypes and does not create new genotypes (see p. 17). A pure line, once isolated, therefore stays constant unless mutation intervenes. Since the success of selection depends upon the availability of a large supply of genes which, by recombination, might produce superior genotypes, one of the powerful breeding methods is *hybridization*. It is evident that if one variety of wheat carries, for example, a superior yielding ability, another variety excels in milling and baking qualities, and still another in resistance to rust fungi, then bringing together into a single genotype the genes for all these desirable properties is advantageous. The best chance of achieving this is by crossing the varieties, raising the F_2 and later generations of hybrids, and isolating among these hybrids the homozygous true-breeding types, pure lines which carry the most desirable combination of genes and of phenotypic traits. Many breeds now in cultivation were obtained by careful selection in the progeny of hybrids. Thus, the English thoroughbred horse resulted from hybridization of Arabian saddle horses with heavier western European riding horses. Thatcher wheat, introduced in commercial planting in 1934 and now grown on many millions of acres in the spring wheat belt of the United States and Canada, is a product of hybridization of three varieties: Iumillo, Marquis, and Kanred. The first of these belongs to the species *Triticum durum* (hard wheat), and it has brought in resistance to stem rust, while the two others are representatives of the soft wheat species *T. vulgare*.

The availability of a supply of genes from which desirable combinations may be constructed is thus the prime concern of a scientific breeder. As pointed out, particularly by Vavilov, this makes imperative the collection, preservation, and study of varieties of cultivated plants and animals, including the most primitive ones, which occur anywhere in the world. For even varieties that by themselves are inferior to the best modern ones may contain desirable genes which can be used to create superior

varieties of the future. Exploitation of the mutation process, particularly of mutations induced by X rays, ultraviolet, and other treatments, is another possible method of procuring new genes for breeding work which until now has been little explored.

Although artificial selection is the most important agent, natural selection is by no means negligible in the evolution of cultivated species. Traits, such as high egg production in poultry or tameness in laboratory mice, which are encouraged by man may be unfavorable for survival and reproduction of the animals under primitive or wild conditions. This consideration makes understandable the tendency of domesticated forms to "degenerate" when artificial selection is relaxed and especially when they escape from cultivation and revert to the wild (feral) mode of life.

Adaptive Changes in Pests and Parasites. Human activities have profoundly altered the environment in which many wild animals and plant species have to live. Many species were unable to become adapted to changed conditions and died out; others adapted themselves very successfully, and became pests, commensals, or weeds. Genetic changes which took place in the process of adaptation are little known, except in a few cases. Thus, at least three species of scale insects which attack the citrus plantations in California have evolved races that are fairly resistant to fumigation with hydrocyanic gas, which is regularly used to control these pests. In the red scale, *Aonidiella aurantii*, the resistance, according to Dixon, is due to a single incompletely recessive gene. Hydrocyanic gas fumigation evidently acts as a powerful selective agent which increases the incidence of the gene for resistance in the populations, leading to the formation of a resistant race which spreads to more and more plantations where fumigation is practiced.

Populations of the stem rust of wheat, *Puccinia graminis tritici*, are mixtures of genotypes of which some are more successful in infecting certain varieties of wheat, while others are more capable of living on other wheat strains. The relative frequencies of rust genotypes in populations change very sharply from year to year, probably in connection with the introduction of new wheat varieties. A wheat variety which is resistant to the particular genotypes of the rust fungus which are prevalent at the time of its introduction may be susceptible to other rust forms. As a new wheat variety is planted more and more widely, the rust genotypes that are able to infect it become correspondingly more widespread.

The form of selection that brings about changes of the kind described in scale insects and rust fungi is properly termed natural rather than artificial selection, since it is brought about not only without man's conscious effort but, in fact, against his will. However, the distinction between natural and artificial selection is to a certain extent arbitrary.

Adaptive Changes in Natural Populations. Apart from changes in nature brought about by man, natural environments in which wild animals and plants live undergo rapid changes from season to season. It is quite evident, at least in countries with a temperate climate, that the environments to which an organism is exposed in spring, summer, fall, and winter are very different. In rapidly breeding organisms, such as certain insects, the alterations which the changing seasons cause call forth genetic responses that are adaptive.

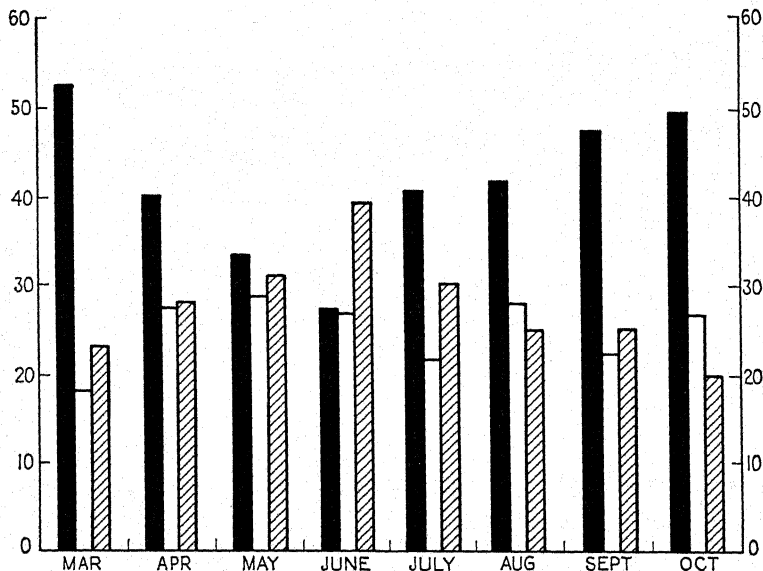


FIG. 143. Seasonal variation in relative frequencies of different inversion types in the third chromosome of *Drosophila pseudoobscura*. Ordinates, frequencies in per cent; black, white, and crosshatched columns represent three different chromosomal types.

Natural populations of many species of *Drosophila* show an interesting variability in chromosome structure. Individual flies may carry chromosomes in which genes are arranged in different orders; the differences between the gene orders are due to inversions of chromosome sections (see p. 258). Thus, at least 12 types of third chromosomes with different gene arrangements occur in populations of *Drosophila pseudoobscura*. A fly may have two third chromosomes either with the same gene arrangement (inversion homozygote) or with different gene arrangements (inversion heterozygote). By examining the chromosomes in salivary glands of larvae in the progeny of flies captured in their natural habitats, it is possible to determine the incidence of different chromosomal types in a given natural population. The population may then be described by the percentages of chromosomes of different types in its chromosome pool.

Studies of this sort have disclosed the fact that, in some populations, the relative frequencies of chromosomes of different types change from season to season. Figure 143 shows the percentages of chromosomes of three types, designated ST, AR, and CH, during different months in the populations of *Drosophila pseudoobscura* in a certain locality in California. It can be seen that ST chromosomes (black columns) decrease in frequency from March to June and increase from June to October. Chromosomes of Type CH, shown in Fig. 143 by hatched columns, become more frequent from March to June and less frequent from June to October. Finally, the frequency of Type AR (white columns in Fig. 143) is rather more constant. A working hypothesis that would explain these changes is that flies which carry ST chromosomes are for some reason more successful in survival and reproduction in summer environments than flies with other kinds of chromosomes, while carriers of CH chromosomes are favored in spring environments. Natural selection augments the frequencies of ST chromosomes in summer and of CH chromosomes in spring.

The validity of the above hypothesis has been tested in several ways. In some of the tests artificial populations of *Drosophila pseudoobscura* were set up in specially constructed population cages, in which the flies could breed in fairly large numbers (several thousands) under conditions of keen competition for a limited amount of food supplied at regular intervals. The initial population of the cage may have any desired proportions of different chromosomal types; from time to time samples of the population are taken, and the frequencies of the chromosomal types in them are determined by analysis of the chromosomes in the cells of the salivary glands. It has been shown by this method that, at temperatures above 25°C. (77°F.), flies homozygous for ST chromosomes have higher adaptive values than AR homozygotes, and these latter have a higher adaptive value than CH homozygotes. Thus, if the initial population of a cage contains few ST but many AR or CH chromosomes, the proportions of ST rapidly rise and those of AR and CH drop, generation after generation. But at temperatures close to 15°C. (59°F.) the adaptive values of the carriers of all chromosome types are equal; hence the proportions present in the initial population persist indefinitely. Thus, at least some of the changes produced by natural selection in wild populations can be reproduced experimentally in laboratory conditions.

In the cage experiments, it is significant that, no matter how long a competition of ST and AR or CH chromosomes lasts, the weaker competitors are never eliminated completely. Instead, an equilibrium is established at which the population contains a greater proportion of ST than of AR or CH chromosomes. The explanation of the failure of natural selection to eliminate the "weaker" chromosomes from the population is that individuals carrying one ST and one AR or CH chromosomes (the heterozy-

gotes ST/AR , ST/CH , and AR/CH) have adaptive values higher than individuals which carry two similar chromosomes (homozygotes ST/ST , AR/AR , and CH/CH). When a heterozygote is superior in survival or reproduction to both homozygotes, the outcome of selection is an equilibrium at which both competing types are retained in the population, although often with different frequencies. This is the explanation of the phenomenon of *balanced polymorphism*, when a species population is a stable mixture of several genotypes. Very striking instances of balanced polymorphism have been described, for example, among some butterflies in which two or more quite differently colored types coexist in the same population (Ford).

Geographic Chromosomal Races in *Drosophila*. Populations of *Drosophila pseudoobscura* which occur in different localities may be described in terms of the relative proportions in them of the different chromosomal types discussed above in connection with the seasonal adaptive changes and experimental populations. The proportions may vary greatly in different localities. For example, ST chromosomes (shown in black columns in Fig. 144) occur frequently in populations of California, but they dwindle in frequency as one moves eastward. AR chromosomes (white columns in Fig. 144) are commonest in Arizona and New Mexico and become less frequent both westward and eastward from there. PP chromosomes (hatched columns in Fig. 144) are commonest in Texas and rapidly become less frequent westward (the species does not occur east of Texas). CH chromosomes (mentioned above but not shown in Fig. 144) are most frequent in populations of northern Mexico and become less frequent as the distance from that region increases.

It can be seen, then, that the differences between populations of different geographic regions are essentially quantitative rather than qualitative; the differences are in relative frequencies of the chromosomal types, although some types may be wholly absent in some localities, as, for example, PP chromosomes, which do not occur in populations of southern California. Moreover, the differences between populations are compounded from the same elements in which individuals within a population may differ, in this case, the gene arrangement in the chromosomes.

Populations of *Drosophila pseudoobscura* which inhabit different territories and which differ in relative frequencies of the chromosomal types may be described as different *races*. Races may, then, be considered as *populations which differ in the relative frequencies of gene alleles or of chromosome structures*.

Race Differences in Man. Human populations native in different parts of the world show racial differences in the incidence of genes for various bodily traits. Owing to the collective efforts of many investigators in

different countries, a very considerable number of data have accumulated on the incidence in human populations of the classical (O-A-B-AB) blood groups, making these by far the best studied genetic trait in the human species. As already stated (pp. 87-90), three alleles of a single gene, I^A ,

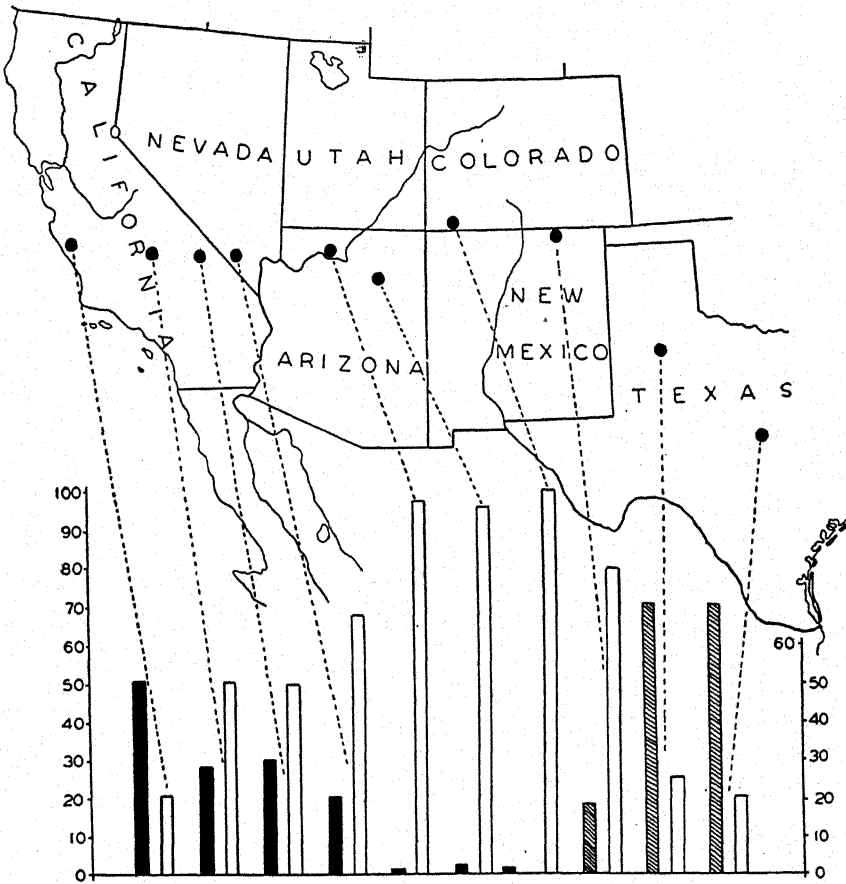


FIG. 144. Relative frequencies of three chromosomal types in geographic races of *Drosophila pseudoobscura* in the southwestern United States. The heights of the black, white, and hatched columns represent the frequencies (in per cent) of the three types.

I^B , and i , account for the inheritance of the principal blood groups. Table XXXIX shows the frequencies of these alleles in certain populations.

It can be seen that, with the exception of some American Indian tribes, populations of all countries contain all three alleles, although with different frequencies. The frequency of I^B (Figs. 145 and 146) is low in west-

ern Europe, increases as one proceeds eastward, reaches a maximum in central Asia and India, and declines again on the shores of the Pacific. Australian Aborigines have very little I^B but considerable I^A . Most American Indians show a very strong predominance of i , amounting in some tribes to exclusion of other alleles, but Blackfeet and related tribes in the northern United States and Canada have very high frequencies of I^A . The maps showing the distribution of these alleles (Figs. 145-148) are based on extensive published statistics for native populations, that

TABLE XXXIX. FREQUENCIES IN THE GENE POOLS OF DIFFERENT HUMAN POPULATIONS OF THE ALLELES i , I^A , AND I^B GIVING RISE TO THE BLOOD GROUPS O, A, B, AND AB (After Snyder and Boyd)

Population	Number of persons tested	i	I^A	I^B
Americans (white).....	20,000	.67	.26	.07
English.....	4,032	.71	.24	.06
French.....	1,197	.62	.29	.09
Germans.....	39,174	.60	.29	.11
Swedes.....	34,000	.61	.31	.08
Hungarians.....	1,500	.54	.29	.17
Hindus.....	2,357	.55	.18	.26
Chinese (Peiping).....	1,000	.54	.20	.26
Koreans.....	9,434	.53	.24	.23
Japanese.....	301,959	.55	.28	.17
Negroes (United States).....	500	.69	.18	.13
Negroes.....	5,000	.74	.14	.12
Hottentots.....	506	.59	.20	.19
Australians (Aborigines).....	805	.73	.26	.01
Indians (Peru).....	200	1.00	.00	.00
Indians (Blackfeet).....	629	.55	.45	.01

is, those which have inhabited these areas since before the time of Columbus.

Differences of similarly quantitative nature between different human populations are also found for other genes determining the immunological characteristics of human blood. The Rhesus blood types (*cf.* p. 90) are particularly interesting. The Rh^0 type occurs in about 40 per cent of American Negroes and in about 4 per cent of Australian Aborigines and is even less common in whites and in the Mongoloids so far investigated. The Rh type is relatively common in whites (12 to 15 per cent) and in American Negroes but is rare in Mongoloids, Australian Aborigines, and American Indians. The Rh_1 type reaches very high frequencies among Papuans, Filipinos, and Indonesians, is common among whites, but is rather less common among American Negroes (African Negroes have not

yet been examined for the Rhesus blood types). The M and N blood types, determined by still another gene, behave similarly. The N type reaches a high frequency among the Australian Aborigines but is infrequent among American Indians and Eskimos, while other peoples show more or less equal proportions of M and N or a slight predominance of the former.

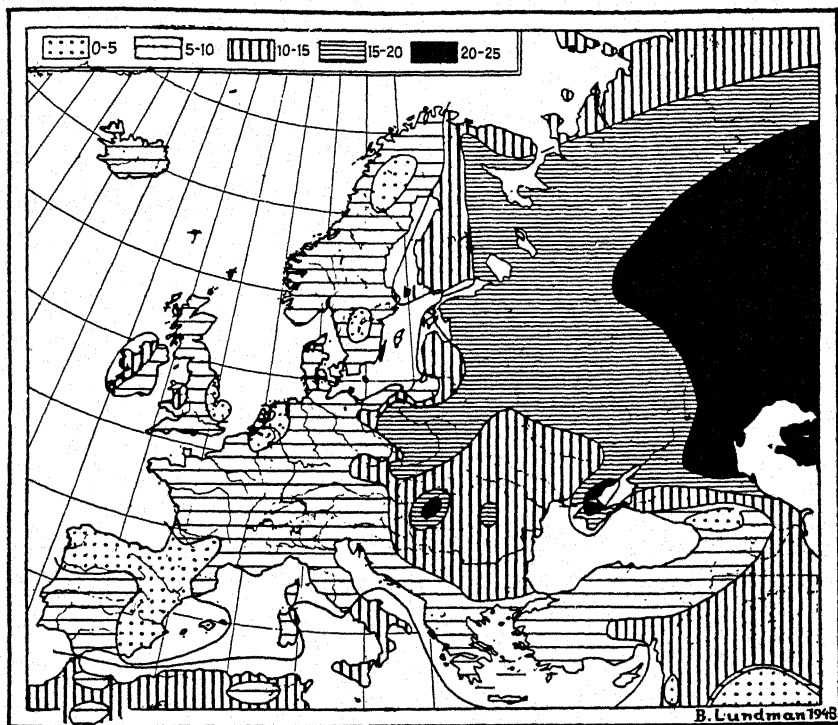


FIG. 145. The distribution of frequencies of the human blood group gene I^B in European populations. (From Lundman.)

The genes that determine the immunological qualities of blood are certainly not the only ones that vary in frequency in human populations. The inheritance of such traits as skin and hair color, stature, head shape, and hair form is not so well known as could be desired. All that can be said at present is that each of these traits is determined by a complex of genes each of which by itself has a small phenotypic effect (polygenes, see Chap. VI). The geographic distribution of these genes cannot be described so accurately as that of the genes responsible for the blood groups. Nevertheless, it is evident from studies on the geographic distribution of the traits themselves that the same principle of racial variation holds for

the blood-group genes and for the genes for visible traits, that is, the differences between human populations are in relative frequencies of certain genes, rather than in the absence in some populations of certain genes that are present in every individual of other populations.

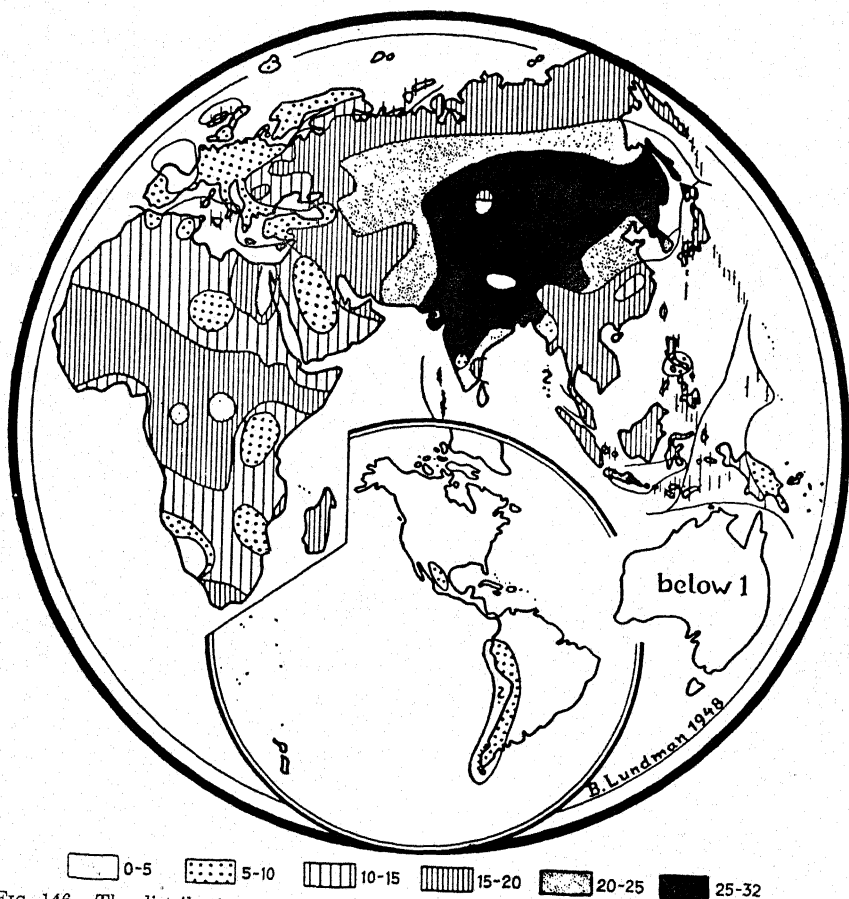


FIG. 146. The distribution of frequencies of the human blood group gene I^B in the world. The frequencies in the Western Hemisphere are those of native (Indian) populations. (From Lundman.)

Genetic Drift. One may nevertheless notice that among the racial traits thus far described there are two categories. Since, in *Drosophila*, the carriers of different chromosomal types have different adaptive values, the chromosomal races found in these flies probably represent adaptive responses of the species to environmental differences in their habitats in different parts of the geographic distribution area of the species. In man,

predominantly dark-skinned populations are found chiefly in countries with intense sunshine and high temperatures and predominantly light-skinned, fair-haired, and blue-eyed populations in countries with extensive cloudiness and a cool climate. Although no conclusive proof is as yet



FIG. 147. The distribution of frequencies of the human blood group gene I^A . The frequencies in the Western Hemisphere are those of native (Indian) populations. (From Lundman.)

available, this correlation between human pigmentation and climate leads one to suppose that dark pigmentation has (or at least had, during the times when human races were being formed) a survival value in sunny and hot areas and light pigmentation in cloudy and cool countries. Other racial traits, however, notably the blood groups in man, do not seem to produce any adaptively valuable or adaptively undesirable traits. Some of the blood groups that differentiate human individuals occur also in anthro-

poïd apes; the blood-group variation was apparently present in the common ancestors of man and apes. The question may then be asked: How can racial variation in adaptively neutral traits arise?

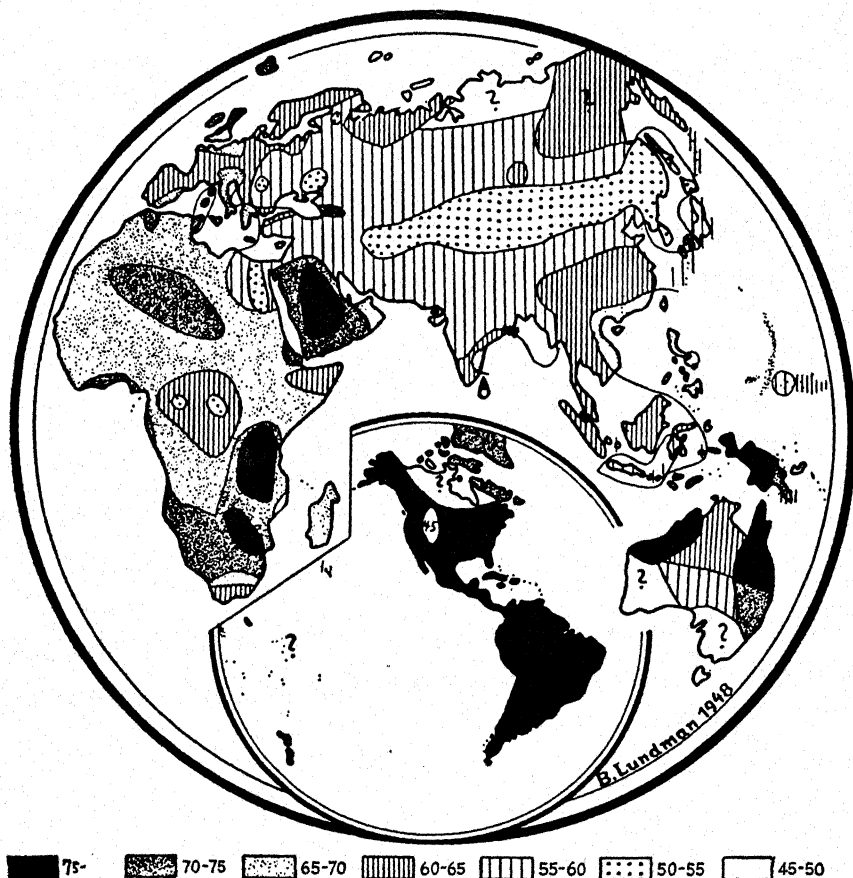


FIG. 148. The distribution of frequencies of the gene i , for O blood group, in man. The frequencies in the Western Hemisphere are those in native (Indian) populations. (From Lundman.)

As shown by Hardy and Weinberg, the frequencies of alleles in the gene pool of a population should remain constant in time, unless mutation, selection, or differential migration alters them (see p. 309). Absolute constancy will, however, prevail only in ideal, infinitely large populations. In reality, populations consist of finite numbers of individuals. Consider, then, two populations consisting of 500,000 and of 50 individuals, respectively. Suppose that in both populations some gene is represented by

two alleles, A and a , which are, to begin with, equally frequent in the gene pool, so that $q = 1 - q = .5$. Now, the larger population arises from 1 million gametes and the smaller one from only 100 gametes. When a sample of 1 million genes is taken from the gene pool which contains equal numbers of alleles A and a , the numbers of A and a will not necessarily be exactly equal; there may be slightly more than 500,000 A and slightly fewer a alleles, or vice versa. According to the formula given on p. 48 the numbers of A and a alleles in a sample of 1 million gametes will be

$$500,000 \pm \sqrt{\frac{500,000 \times 500,000}{1,000,000}} = 500,000 \pm 500$$

Similarly, in the small population, the expected numbers of A and a alleles in a sample of 100 gametes will be

$$50 \pm \sqrt{\frac{50 \times 50}{100}} = 50 \pm 5$$

The standard error for the large population is 500 and for the small one only 5. However, for the large population the standard error constitutes only .1 per cent of the gamete number, while in the small population it constitutes as much as 10 per cent of the gamete number. It is easy to see, then, that the proportions of the alleles A and a in the gene pool will remain rather constant from generation to generation in the large population, while they will be much more variable in the small population. Variations in gene frequencies which arise because of sampling errors in finite populations are known as *genetic drift*. Sewall Wright has shown, by mathematical analysis, that, if several small populations have at some time identical gene frequencies, these gene frequencies may, because of genetic drift, become different with time. Similarly, in any one population, the gene frequencies may change with time if the population is small.

Genetic drift may, then, bring about differences in the frequencies of adaptively neutral genes among populations, provided that these populations are small. Of course, the smaller the population, the greater will be the importance of genetic drift in them (the "bottleneck" effect), while in very large populations it will be negligible. Whether or not the differences in the incidence of the blood-group genes observed among human populations can be accounted for by genetic drift alone is a problem that cannot be settled at present. This depends on whether or not human populations were sufficiently small to permit the requisite amount of genetic drift to occur during the early stages of human evolution, when the human species was becoming distributed over the face of the earth. More studies in this field are needed.

Races and Individuals. In organisms that reproduce sexually and by cross-fertilization, races are populations which differ in the relative frequencies of genes or chromosomal structures. This genetic conception of race emphasizes the fact that races are units which describe neither individuals nor arbitrarily chosen groups of individuals, but populations, that is, breeding communities the members of which share in a common gene pool. The following example will illustrate the import of this distinction.

It may seem that it would be reasonable to designate everybody having blood of group O as belonging to an "O race," everybody with blood of group A as belonging to an "A race," etc. The race to which any individual belongs would then be determinable simply by testing his blood. Such a "racial" classification would, however, lead to absurd consequences, because brothers and sisters often have bloods of different types, and children often have bloods different from their parents. Furthermore, a "race" classification built on the O-A-B-AB blood grouping would not coincide with that built on the basis of the Rhesus alleles, while the eye colors, skin colors, head shapes are again distributed independently of the blood groups and of each other. In other words, a "race" classification that would unite in a single race individuals with identical genotypes might be applicable only in organisms which reproduce asexually or by self-fertilization, in which clones and pure lines exist, the members of which are genotypically identical. But in cross-fertilizing organisms such a classification would be absurd, because virtually every sexual individual has a genotype not found in any other individual, so that a "race" would always consist of a single individual.

Since members of a race in crossbreeding organisms have different genotypes, it may happen that an individual of one race population will, with respect to some genes, resemble members of another population more than it resembles other individuals of its own race. For example, a "white" person who has blood of Rh⁰ type resembles, *to that extent*, many members of the Negro race (see p. 342); and an individual of *Drosophila pseudoobscura* from Arizona carrying an ST chromosome is, in that respect, more like a representative of the California population than most other individuals native in Arizona (Fig. 144, p. 341). Because races are populations and not individuals, it may be difficult or impossible to classify some individuals as belonging to any one race. Thus a specimen of *D. pseudoobscura* with PP chromosomes comes probably from Texas rather than from California, but a specimen with AR chromosomes may occur anywhere from Texas to California, (Fig. 144). It is easy to distinguish members of the human population native in northern Europe from members of the population of central Africa; but members of the northern European and central European populations are often indistinguishable.

Race Divergence. Race differences are of different magnitudes. Populations of different territories may contain the same genic or chromosomal variants with only slightly different frequencies, so that careful statistical studies of large samples of these populations may be needed to distinguish them at all. Such are the human populations of northern and central Europe or populations of *Drosophila pseudoobscura* from different parts of California (Fig. 144). Or the gene frequencies may be distinct enough to enable identification of most individuals as belonging to one or the other population, yet every gene allele or chromosome structure may occur in both populations, although with different frequencies. This is the case with human populations native in Europe and in Africa or populations of *D. pseudoobscura* in California and in Arizona. Finally, some genes, or groups of genes, or chromosomal structures may be present in every individual of one population but wholly absent in individuals of another population, such as the *D. pseudoobscura* populations of Arizona and southern Mexico.

Of course, in systematic zoology and botany only populations that are distinct enough so that at least 80 per cent of the individuals in them are classifiable as belonging to one or the other population are referred to as races and are given scientific names in Latin (Mayr). But geneticists and evolutionists will be inclined to emphasize that races may have any degree of distinctness. Indeed, races are dynamic and not static entities; they become more and more distinct in the course of evolution and finally cease to be races and turn into separate species. This is an essential part of the theory of evolution advanced by Darwin and accepted by most other evolutionists. Populations living in different countries with different environments become progressively more and more adapted to these environments and accumulate more numerous and more profound genetic differences. As shown in Chapter VI, races of a species may differ in many genes, just as species do.

It is a fairly general rule that races of a sexually reproducing species occur in different territories, so that in no one territory will more than a single race of any one species be found. In other words, most races are *geographic races* or *subspecies*. This is easily understandable, for if more than a single sexual population were placed in the same territory, members of these populations would cross, and the resulting gene exchange between the populations would make them genetically similar and would lead to their fusion into a single population. This is indeed what happens when man brings representatives of different races of animals or plants in the same territory and fails to take measures to prevent their interbreeding.

The rule of geographic separation of races of a species has important

exceptions. One of these exceptions is the human species. The development of culture permitted human races, which were in the remote past restricted to different countries, to exist side by side in the same geographic region without immediate fusion. This was possible because intermarriage of members of different human populations, or *isolates* as they have been called by Wahlund and Dahlberg, was often prevented not only by geographical but also by social, religious, economic, and other cultural causes. Nevertheless, it is virtually certain that the genetic differences between human populations are decreasing rather than increasing, so that the isolates become gradually broken down and fused into larger more or less panmictic populations.

Varieties and "breeds" of domesticated animals and plants are also races in the genetic sense, some of which occur in the same territory without fusion. Thus, several breeds of dogs, including the remarkably variable breed called the mongrel, occur in any American town. It is, however, evident that the separate existence of these breeds is possible only so long as interbreeding between representatives of different breeds is prevented by man, who accomplishes for the breeds of dogs what is performed for races of wild animals and plants by geographic isolation. The care exercised by agriculturists to prevent interbreeding of cross-pollinated plant varieties also permits these varieties to be planted in the same geographic region, although still at a certain distance from each other. The situation is quite different for asexually reproducing or self-pollinated crops, for in them relatively simple precautions suffice to prevent contamination of the genotypes of different populations.

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PROBLEMS

314. Give some reasons why animals and plants under domestication are more variable than corresponding wild species.

315. Assume that a sexually reproducing population remains stationary in numbers generation after generation, and that every pair of parents produces two surviving children. In a certain generation there appears a single mutant individual which is neither superior nor inferior in adaptive value to the prevailing condition. What is the chance that this mutant will be present in one individual in the next generation? That it will be lost? That two mutant individuals will appear?

316. Suppose that the population is like that described in Problem 315, but that some parents leave no surviving progeny at all and other parents leave one, two, or several offspring. What are the chances of loss, of retention, and of increase in frequency of an adaptively neutral mutant in such a population?

317. Only a fraction of the human beings who lived in the year 2,000 BC are the actual ancestors of the men now living. Ignoring the occurrence of selection and of mutations, what has happened to the individual genes that were present in the human population of the world in 2,000 BC?

318. Compare the fates of individual human genes that were present in the populations that lived in 1,000 AD, 2,000 BC, and 10,000 BC.

319. Is selection for a dominant trait, other things being equal, more or less effective than selection for a recessive trait? Why?

320. Suppose that an autosomal and a sex-linked recessive mutant gene is each equally deleterious to its carrier. With equal mutation rates, which of these mutants will be more frequently encountered in a sexual cross-fertilizing population?

321. The relative frequencies of the blood groups O, A, B, and AB are different in human populations of different parts of the world. What working hypotheses can you suggest to account for these different frequencies?

322. Melanic (darker colored) variants have appeared in several species of moths in England and Germany, nearly always first near large industrial cities, where in some cases they have supplanted the normal type of the species. Assuming that the dark and light types differ in one or a few genes, outline an explanation for the above facts, together with suggestions for testing the hypothesis experimentally.

323. The frequency of a Race A of wheat rust increased manyfold in the United States during a period when the frequency of a related Race B of the same species declined and became nearly extinct. Suggest explanations which could be tested.

CHAPTER XIV

GENETICS OF SPECIES FORMATION

Species are fundamental biological units which, before the advent of evolution theories, were regarded as originating in separate acts of creation. How great an importance was attached by Darwin to the demonstration that species arise by divergence of races of previously existing species can be seen from the title of his classical work, "The Origin of Species." Genetics, particularly in recent years, has made significant contributions toward a better understanding of the nature of species and of the mechanisms which bring about the emergence of species from races.

Reproductive Isolation and Speciation. We have seen in the preceding chapter that races of sexually reproducing species are, as a rule, geographically isolated, that is, segregated in different territories. Geographic isolation permits races not only to maintain their distinctness as populations but also to accumulate more and more genetic differences. Genetic divergence may eventually transform races into *reproductively isolated populations*, that is, into species. Owing to reproductive isolation, related species may, and often do, occur side by side in the same territory. Transformation of races into species consists, then, in the development of reproductive isolating mechanisms between the diverging populations. A *reproductive isolating mechanism* is any genetically determined agency which restricts or prevents the interbreeding of natural populations. Several kinds of reproductive isolating mechanisms are known. The following are probably the most important in the maintenance of species in nature.

Populations of different species may be confined to different habitats in the same geographic region because of their preferences for different soils, or different amounts of moisture, or association with different hosts or food sources. Such *ecological isolation* may result in little or no opportunity for members of the isolated populations to meet and hybridize. For example, the spiderwort species *Tradescantia canaliculata* and *T. subaspera* in the Ozark region of Missouri grow, respectively, on sunny exposures at the tops of cliffs and in the forest shade at the bottom of cliffs (Fig. 149). In the Sierra Nevada of California, the related species *Drosophila pseudoobscura* and *D. persimilis* prefer, respectively, the low and the high elevations.

If representatives of different species reach sexual maturity or flowering

times at different seasons of the year, they are called *seasonally isolated*. According to Blair, the toad *Bufo americanus* breeds earlier in the spring than the related species *B. fowleri*, although the breeding seasons of the two species overlap to some extent.

When females and males of different species come together, matings may occur mainly or exclusively between representatives of the same species. This is *sexual isolation*. For example, if a mixture of females of *Drosophila persimilis* and *D. pseudoobscura* are exposed to males of one of these species, the proportion of conspecific females that are inseminated is greater than that of foreign females. In most instances such selective mating occurs because courtship and mating habits often differ in different species. It may occur also because the *genitalia* of one species are

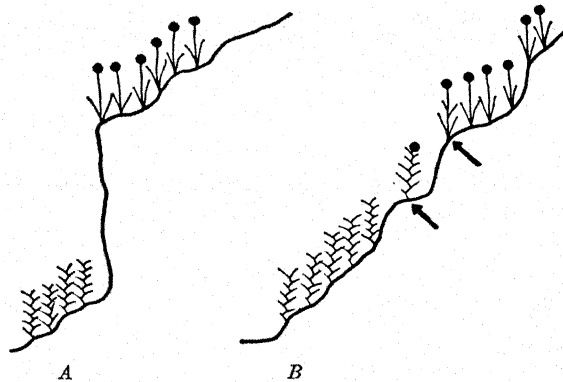


FIG. 149. Ecological isolation of two spiderworts. *Tradescantia canaliculata* grows on cliff tops, and *T. subaspera* at the foot of cliffs (A). In some places, where the slopes are more gentle (B), the two species come together and hybridize in the intermediate habitat. (After Anderson and Hubricht.)

mechanically incompatible with the genitalia of the opposite sex of another species (*mechanical isolation*). In plants, mechanical isolation manifests itself in differences in flower structure which make cross-pollination by the same insect difficult.

Spermatozoa of one species may not be attracted to eggs of another or may be poorly viable in the sexual ducts of females of foreign species (*gametic isolation*). Thus, spermatozoa of *Drosophila virilis* survive for only about a day in the sperm receptacles of *D. americana* females, while they live for weeks in the receptacles of conspecific females. In plants, the pollen tubes of one species may grow slowly, or may burst, in the styles of other species, as shown in species of the Jimson weed (*Datura*) by Blakeslee, Buchholz, and collaborators.

If F_1 generation hybrids between species are produced, gene exchange be-

tween the populations of these species may nevertheless fail to occur because of *hybrid inviability* or *hybrid sterility*. Hybrid zygotes may die at any stage, from the time immediately following fertilization to reproductive maturity. For example, the two species of flax, *Linum austriacum* and *L. perenne*, can be crossed, but the hybrid seeds fail to germinate. Laibach showed, however, that if the embryos are dissected from these seeds and grown on nutrient media they eventually give rise to seedlings, which then develop into normal plants which are fertile. In other species crosses the hybrids not only survive but may be fully as vigorous as the parental species, and yet they may be partly or completely sterile. The classical example of hybrid sterility is the mule, which is the outcome of hybridization of horse and ass. The degeneration of the sex cells in mules contrasts strongly with their general hardiness and normality of sexual instincts. Female mules are alleged to be occasionally fertile. Fertility of one sex in species hybrids frequently goes together with partial or complete sterility of the other sex. Thus, *Drosophila pseudoobscura* and *D. persimilis* produce completely sterile male hybrids, while hybrid females deposit about as many eggs as do females of pure species when crossed back to males of either of the latter.

Since species usually differ in many genes, progenies of fertile interspecific hybrids frequently show segregations so complex that no two individuals in them look alike or carry similar genes. The genotype of each species which exists in nature represents an at least tolerably harmonious combination of genes which enables the species to survive. The gene recombinations that arise in species hybrids are, on the contrary, often disharmonious to such an extent that the F_2 of species crosses may consist largely of ill-adapted individuals. Such a *hybrid breakdown* has been observed, for example, in hybrids between different species of cotton (*Gossypium*) by Harland, Stephens, and other investigators.

Partial and Complete Isolation. It must be emphasized that any of the reproductive isolating mechanisms may be either complete or partial. Thus, sexual isolation may amount merely to a slight preference for mating with representatives of the same species. It may be strong enough so that mating of females of one species with males of another occurs only as an exception. Or the aversion to interspecies mating may be absolute. Hybrid inviability may involve only a constitutional weakness or the hybrids may seldom or never survive.

Furthermore, the exchange of genes between related species is usually prevented not by a single isolating mechanism but by cooperation of several mechanisms which reinforce one another. For example, although *Drosophila pseudoobscura* and *D. persimilis* show some ecological isolation, both species occur side by side in many localities; sexual isolation is quite pro-

nounced, and yet if females of one species are confined with males of the other, some cross-inseminations occur; male hybrids are invariably and completely sterile, but hybrid females are fertile if crossed back to males of the parental species; the backcross products are weak, but some individuals survive. In laboratory experiments, genes and chromosome sections of either species may, by appropriate crosses, be "transferred" to the other species. Nevertheless, no hybrids between these species have been found in nature, even in localities where they occur together, and other evidence shows that such hybrids must be very rare, if they occur at all.

The combined actions of several reproductive isolating mechanisms may add up sometimes to a complete suppression and sometimes to only a partial inhibition of hybridization and consequent gene exchange between species populations. Interspecific hybrids are known to occur in nature as more or less exceptional individuals. Thus, Blair finds that hybrids of the toads *Bufo americanus*, and *B. fowleri* occur rather regularly whenever these two species inhabit the same territory.

Attempts have often been made to define species as forms which produce no hybrids at all or else produce completely sterile hybrids. All such attempts break down. There exist species which are completely isolated reproductively, so that no hybrids occur in nature, and yet they can be crossed and produce fertile hybrids in experiments (for example, the Mallard and Pintail ducks, *Anas platyrhynchos* and *Dafila acuta*). Furthermore, since species arise by gradual divergence of races, there is bound to be a stage in this divergence process when some reproductive isolation has appeared but when it is not yet strong enough to suppress the gene exchange to an extent sufficient to maintain the species populations as separate entities. In other words, there exist borderline cases between races and species. Patterson and his collaborators regarded *Drosophila americana*, *D. texana*, and *D. novamericana* as distinct species but, having found some hybrids between them in nature, now prefer to consider them very distinct races of a single species. The existence of such borderline cases is troublesome to taxonomists, who would like to have races and species absolutely fixed categories, but such cases are most interesting and valuable to a geneticist, since they furnish one of the best proofs of the reality of the evolutionary process.

The attainment of the species status, that is, the advent of reproductive isolation between populations, is, biologically considered, an event of fundamental importance. This is because the evolutionary divergence becomes irreversible at this stage. So long as diverging races are not yet reproductively isolated, they are potentially able to hybridize and to merge back into a single population. Human races are an excellent example of such a merging process. Even before the dawn of civilization *Homo sapiens* had become split up into races which, if the genetic divergence had con-

tinued, might have reached the status of separate species. But with civilization came progressively increasing mobility of human populations so that the races of man now show an unmistakable trend toward hybridization and fusion into a single highly variable population. In contrast to the reversal of the racial differentiation in man, horse and ass are species which have attained complete or nearly complete reproductive isolation. Accord-



FIG. 150. Typical representatives of maize (left) and of *Tripsacum* (right). (Courtesy of P. C. Mangelsdorf.)

ingly, a genetic barrier between them is fully maintained despite the mass production of man-made hybrids which now occur together with the parent species. Some or all of a group of related intersterile species may become extinct, but they cannot become fused into a single population.

Introgressive Hybridization. Results of great interest for genetics may be produced by occasional hybridization of incompletely isolated species. In nature, highly variable populations which consist of segregating progenies of species hybrids (*hybrid swarms*) may occur at geographic boundaries which separate the distribution regions of related species, especially

in plants. Thus, the ecologically isolated species *Tradescantia canaliculata* and *T. subaspera* (see p. 354) intercross in places where their habitats merge into each other.

Teosinte (*Euchlaena mexicana*), a grass species found in some parts of Mexico and Guatemala, is, botanically speaking, the nearest relative of cultivated maize, *Zea mays*, growing in a wild state (Figs. 150 and 151). It



FIG. 151. A plant of teosinte (*Euchlaena mexicana*). (From Mangelsdorf and Reeves.)

was consequently believed to represent the wild progenitor from which maize arose in cultivation. Mangelsdorf and Reeves have, however, put forward an alternative hypothesis, according to which teosinte, far from being the ancestor of cultivated maize, is itself the result of hybridization of maize with a still different grass, namely, *Tripsacum*. The chromosomes of teosinte are mostly similar to those of maize, except for four different blocks of genes, which are presumably derived from *Tripsacum*. Maize arose, according to this view, from an unknown South American plant. As maize became introduced into Central America for cultivation, it encoun-

tered *Tripsacum*, and from hybridization of the two there arose, on the one hand, teosinte and, on the other, the modern varieties of maize now cultivated in North America. Hybridization which introduces some genes of one species into the genotype of another species has been called by Anderson *introgressive hybridization*.

Morphological and Genetical Differences between Species. Races of a species and different species of a genus carry different complexes of genes which make them best fitted to survive and reproduce in different habitats. As a rule, the genetic differences manifest themselves in differences in color, size, and properties of various body parts, and these external manifestations of the genotypic differences permit recognition of every individual as belonging to a certain species and sometimes as belonging to a certain race of a species. Systematic zoologists and botanists are hence justified when they characterize species and races by their visible external characteristics. The genetic divergence need not, however, be strictly proportional to the externally visible divergence. This leads to exceptional situations, when species which are reproductively completely isolated are similar or identical in appearance, or when races of a species have strikingly different forms. A striking example of species which are morphologically indistinguishable are *Drosophila pseudoobscura* and *D. persimilis*. The two have different geographical distributions, different habitat preferences, and many physiological differences, and reproductive isolation between them is strong enough so that no hybrids are found in natural populations. They differ also in the shape of the Y chromosome and in inversions of blocks of genes in three of the five chromosomes. Yet except for very slight and broadly overlapping differences in the proportions of the wings and of the thorax the two species are morphologically identical. Conversely, geographic races within certain species of pheasants, birds of paradise, and insects show external differences which are more striking than those which distinguish some species.

Genetics of Reproductive Isolating Mechanisms. The process of species formation consists, then, in the development of reproductive isolation between diverging populations. Isolating mechanisms, in turn, are determined by differences in genes and in chromosome structures, and these arise ultimately by mutation. A single example of a gene difference which causes reproductive isolation will suffice.

Hollingshead found that, in populations of *Crepis tectorum*, a plant belonging to the family Compositae (Fig. 152), some plants carry a dominant gene, *T*, which is lethal in hybrids with the related species, *C. capillaris*. If an individual of *C. tectorum* is homozygous for *T* (*TT*), all hybrids between this individual and *C. capillaris* will die in the seedling stage. If a *Tt* heterozygote of *C. tectorum* is used as the parent, only half of the hybrids

with *C. capillaris* die, namely, those which inherit the gene *T* from the *C. tectorum* parent. Finally, if a *tt* individual of *C. tectorum* is used, the hybrids survive. The alleles *T* and *t* seem to have no visible effect in pure *C. tectorum*, so that *TT*, *Tt*, and *tt* individuals are indistinguishable, except when crossed to *C. capillaris*. It is easy to imagine that if the whole population of *C. tectorum* were to become homozygous for the allele *T*, the two species would be completely isolated from each other by the inviability of their hybrids. However, it should be noted that the death of the hybrid seed



FIG. 152. Individuals of *Crepis tectorum* (left), *Crepis capillaris* (right), and their viable hybrid (center). (From Hollingshead.)

lings cannot be ascribed to the gene *T* alone but to interaction of *T* from *C. tectorum* with some other gene or genes coming from *C. capillaris*. This follows clearly from the fact that in pure *C. tectorum* the gene *T* has no detectable effects. In general, reproductive isolating mechanisms which act as barriers for gene exchange between species are probably always caused by interaction of two or more complementary genes contributed by the species crossed. When species arise from races, one gene (or group of genes) which eventually produces isolation becomes established in one race and the complementary gene (or genes) in another race. These races or incipient species may now coexist in the same territory because they are re-

productively isolated and cannot exchange genes. This is probably the reason why, in sexual and cross-fertilizing organisms, two species cannot arise from a single ancestral species except through divergence of geographic races. As Mayr has emphasized, geographic isolation of races is a necessary stage of speciation.

The genetics of hybrid sterility has been studied more than that of any other isolating mechanism. In many instances the sterility proved to be caused by abnormal behavior of chromosomes at meiosis in interspecific hybrids.

Differences between Species in the Number and Form of Chromosomes.

Species of a genus or a family may have either similar or different numbers of chromosomes. Thus, most of the numerous species of several subfamilies of short-horned grasshoppers (Acrididae) have 12 pairs of chromosomes. Mosquitoes have 3 pairs of chromosomes. On the other hand, species of *Drosophila* may have 3, 4, 5, or 6 chromosomes, and closely related species often have different numbers. The Old World species of *Crepis* (plants of the family Compositae) have 3, 4, 5, 6, or 7 chromosomes, while American species have 11 or more chromosomes. A special case of variation in chromosome numbers occurs in polyploid series, the members of which have chromosome numbers that are multiples of some basic number. For example, species of wheat and related grasses have 7, 14, or 21 chromosomes. Polyploid series are common in some genera of plants but rare in animals.

Except for polyploidy (discussed on p. 371) changes of chromosome numbers arise by translocations. Suppose, for example, that two of the chromosomes in a haploid set of a certain species have subterminal centromeres (Fig. 120, p. 26). A translocation shown in the diagram results in formation of a V-shaped chromosome (with a median centromere) and a small fragment with another centromere. If this fragment is subsequently lost, the chromosome number becomes one less than it originally was, and yet few or no genes are lost since the part of the chromosome adjacent to the centromere is often heterochromatic and contains few indispensable genes. Increase of the chromosome number usually requires a duplication by non-disjunction of a chromosome or a chromosome fragment to furnish an extra centromere. A translocation of a part of one of the other chromosomes onto the duplication then gives a chromosome group (*karyotype*) with one chromosome more than was originally present (Fig. 111, p. 251). Dubinin has succeeded in proving that these theoretical schemes actually work in practice; he obtained in experiments a strain of *Drosophila melanogaster* with *three* instead of the original *four* pairs of chromosomes. Less direct evidence has been obtained by Tobgy for *Crepis fuliginosa*, with three pairs of chromosomes, and *C. neglecta*, with four pairs of chromosomes (Fig. 153).

These changes in the numbers of chromosomes are made possible by the

fact that in the great majority of plants and animals each chromosome has a specialized organelle, the centromere (p. 262), which controls the anaphase movements of the chromosomes. Chromosomes which lack a centromere undergo irregular disjunction at mitosis, fail to be included in the daughter nuclei, and ultimately are lost. In some plants at least, the centromeres are capable of being fractured, and it is a remarkable fact that each portion of the original centromere is still able to function normally and thus to provide one additional centromere. The divisibility of the centromeres has made it possible for Rhoades to synthesize a strain of maize with 11 rather than 10 pairs of chromosomes. It is possible that increases in chromosome number in the course of evolution have occurred in a similar fashion.

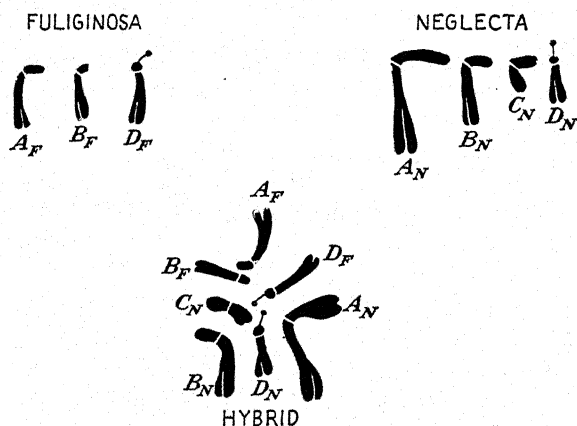


FIG. 153. Haploid sets of chromosomes of *Crepis fuliginosa* and *Crepis neglecta*, and a diploid chromosome group in a hybrid of these species. (From Tobgy.)

A convincing demonstration of evolutionary changes in karyotype brought about by translocations has emerged from comparative cytogenetic studies on species of *Drosophila*. The simplest, and perhaps ancestral, chromosome complement present in species of various sections of the genus *Drosophila* consists of five pairs of rod-shaped and one pair of dotlike chromosomes (*Drosophila subobscura*, *D. virilis*, and others, Fig. 154). One of the rod-shaped chromosomes is the X chromosome. Translocation between the X chromosome and one of the autosomes gives rise to the chromosome complement found in *D. pseudoobscura* and *D. persimilis*: a pair of V-shaped X chromosomes and three pairs of rodlike and a pair of dotlike autosomes. Two more translocations combine two of the autosomal rods into another V-shaped autosome with a median centromere and cause the dot to join the remaining rod, thus giving rise to the karyotype of *D. willistoni* (Fig. 154). The karyotype of *D. melanogaster* is derived from

the presumed ancestral one by two translocations which tie together four rod-shaped autosomes to become two V-shaped ones, leaving the rod-shaped X chromosome free. Thus, most of the genes that are borne in the rod-

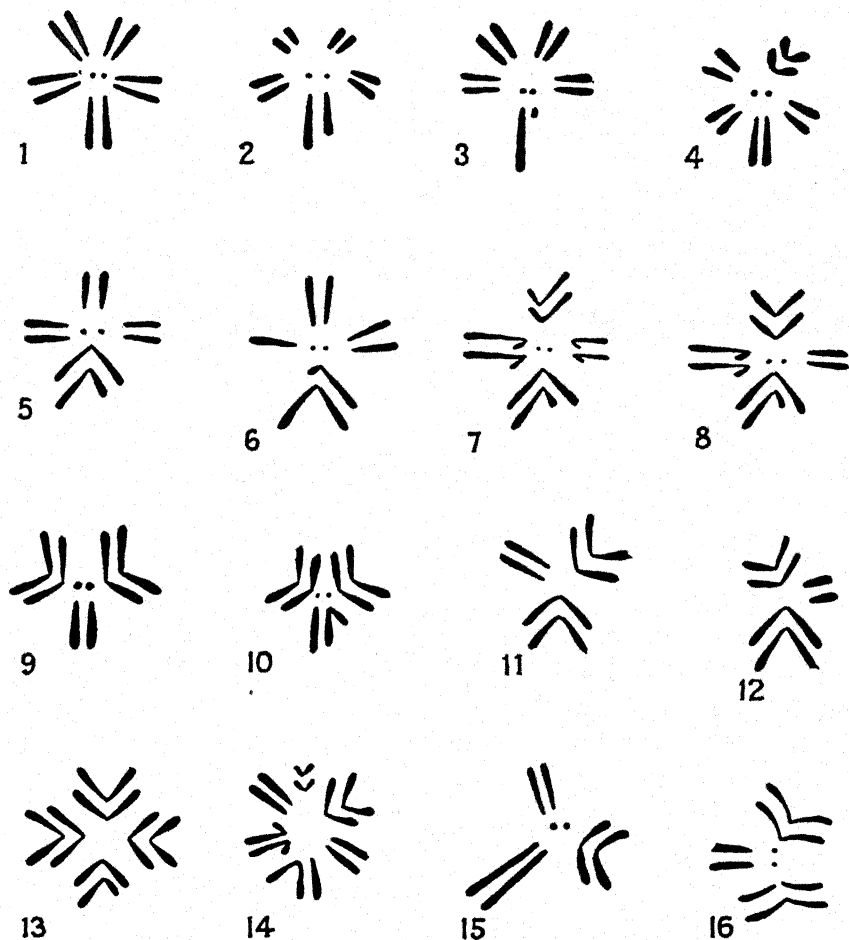


FIG. 154. Chromosome complements in males of some species of *Drosophila*. The drawings are so oriented that the X and Y chromosomes (which are visibly different in some species but not in others) are in the lower portion of each drawing. 1, *Drosophila virilis*; 2, *D. funebris*; 3, *D. repleta*; 4, *D. montana*; 5, *D. pseudoobscura*; 6, *D. miranda*; 7, *D. azteca*; 8, *D. affinis*; 9, *D. putrida*; 10, *D. melanogaster*; 11, *D. willistoni*; 12, *D. prosaltans*; 13, *D. ananassae*; 14, *D. colorata*; 15, *D. spinofemora*; 16, *D. americana*. (After Wharton.)

shaped X chromosome of *D. melanogaster*, *D. subobscura*, and *D. virilis* lie in just one of the two arms of the V-shaped X chromosome in *D. pseudoobscura*, *D. persimilis*, and *D. willistoni*. The genes in the other arm of the

X in the three last-named species, which behave in inheritance as sex-linked genes, are borne in autosomes in *D. melanogaster* and *D. subobscura* and in these species behave as regular autosomal genes.

Among plants, translocations must have taken place in the evolution of races of the Jimson weed, *Datura stramonium*, as well as of species of the genus *Datura*. Blakeslee and his collaborators observed rings of chromosomes, characteristic of translocation heterozygotes (cf. pp. 252-255), in hybrids of *Datura* races and species. They were able to determine just which chromosomes of a standard strain of *D. stramonium* are involved in the translocations which characterize different races of this species and different related species. Babcock and his school have carried out a painstaking study of the evolution of the karyotype in species of the genus *Crepis* and showed that translocations have been instrumental in bringing about variations in the chromosome numbers in this genus.

Inversions and Chromosomal Differentiation of Species. Inversions of blocks of genes play an important role in the evolution of chromosomes. However, changes of the gene arrangement produced by inversions only rarely lead to visible alterations of the chromosome shape, when in some pericentric inversions (see p. 260) a shift in the position of the centromere occurs with respect to the ends of the chromosome. An originally V-shaped chromosome (with a median centromere) may then become hook-shaped or rod-shaped (with a subterminal centromere (Fig. 120, p. 261), or vice versa. Such changes have been observed in the offspring of *Drosophila* treated with X rays, and comparison of chromosome shapes in different species shows that pericentric inversions have been active in the evolution of this genus. Similar evidence exists for grasshoppers and, less directly, for some other animals and plants.

Inversions of chromosome sections which do not include the centromere, that is, paracentric inversions (p. 259) lead, as a rule, to no changes in the appearance of chromosomes at metaphase of mitosis. Accordingly, such inversions are detectable only in forms which are exceptionally favorable for cytogenetic studies. Discovery of the giant chromosomes in the cells of larval salivary glands has permitted the detection of numerous inversions in natural populations of several species of *Drosophila* and of some other flies (pp. 338 and 341). Observations on salivary-gland chromosomes in hybrids between closely related species of *Drosophila* have disclosed that inversions have played a relatively more important role in the evolution of some species than in that of others. *Drosophila melanogaster* and *D. simulans* are species that are rather similar morphologically but form completely sterile hybrids. The chromosomes of hybrid larvae show only a single large inversion in one of the autosomes and several small changes in the gene arrangement in other chromosomes. *Drosophila pseudoobscura* and *D.*

persimilis are morphologically very nearly identical and form hybrids which are sterile in the male sex but fertile in the female. Their chromosomes differ usually in four large inversions.¹ The incipient species or races *D. americana*, *D. texana*, and *D. novamexicana* and their close relative, *D.*

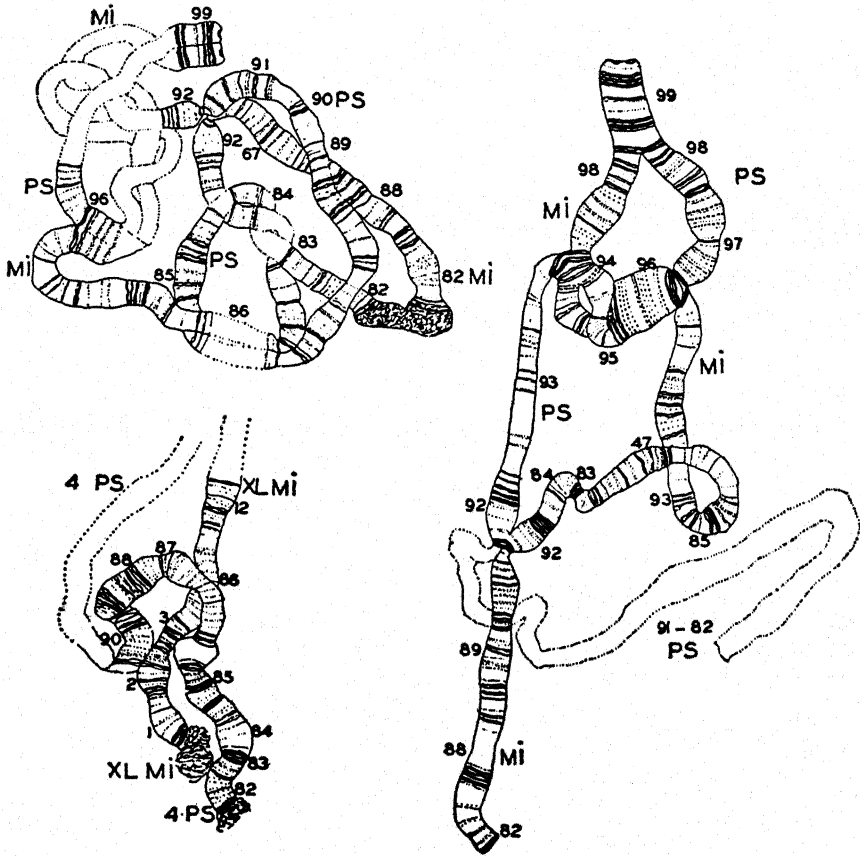


FIG. 155. Pairing configurations in salivary-gland chromosomes of hybrids between *Drosophila pseudoobscura* and *D. miranda*. (After Dobzhansky and Tan.)

virilis, show slight morphological differences and give semifertile hybrids. According to Patterson and his collaborators, these forms differ in up to eight inverted sections in several chromosomes. Finally, *D. pseudoobscura* and *D. miranda* differ morphologically to about the same extent as *D.*

¹ Since, as shown above, inversions occur not only between species but also within a species, the number of inversions in interspecific hybrids depends somewhat on which strains of the parental species are used to produce hybrids.

melanogaster and *D. simulans* and also give completely sterile hybrids. The gene arrangements in their chromosomes have been modified by repeated inversions to such an extent that the homology is no longer recognizable in many chromosome sections, and very complex pairing configurations are formed by the chromosomes in the salivary-gland chromosomes of hybrid larvae (Figs. 155 and 156).

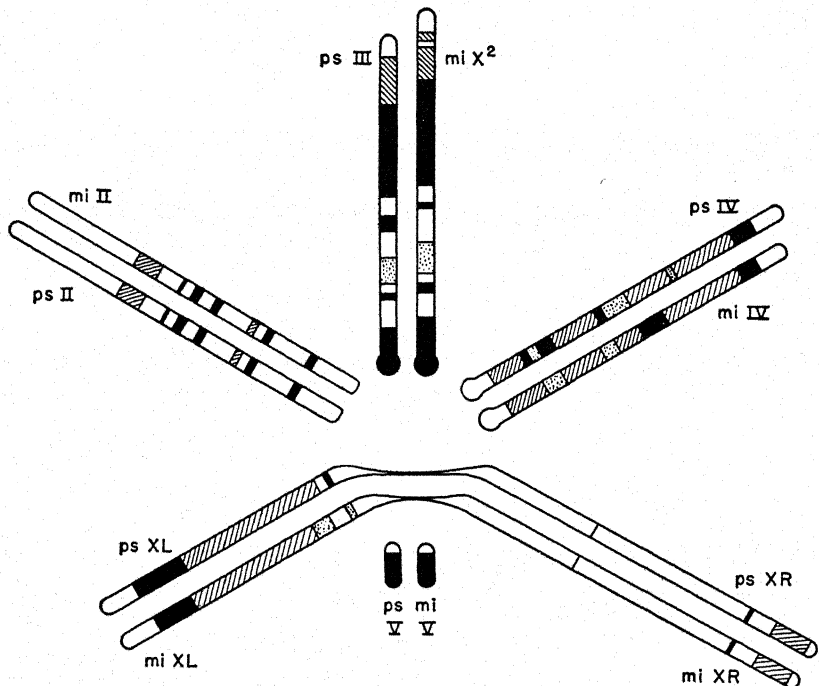


FIG. 156. A comparison of the chromosomes of two related species, *Drosophila pseudoobscura* (ps) and *Drosophila miranda* (mi). Sections in which the gene arrangements are similar in the two species are shown in white; sections which were displaced within a chromosome by inversions are crosshatched; sections which were shifted between chromosomes (by translocations) are stippled; and sections which have not been identified in the other species are black. (After Dobzhansky and Tan.)

It can be seen from the foregoing paragraphs that the degree of morphological divergence, the degree of hybrid sterility, and that of the differentiation of the chromosome structure do not necessarily go hand in hand in evolution.

Genic and Chromosomal Sterility. In 1913, while studying spermatogenesis in the semisterile hybrids between species of the moth genus *Pygaera*, Federley made the fundamental discovery that the sterility is connected with the failure of the chromosomes of the parental species to

pair at meiosis. In the testes of the hybrid males, the behavior of the cells is normal up to the stage when the chromosomes should undergo pairing. Only a few of the chromosomes do pair, whereupon most of the prospective sex cells degenerate and make the hybrid very nearly sterile. Failure of the meiotic pairing of chromosomes has been observed since 1913 in many other semisterile and sterile species hybrids both in plants and in animals. This naturally leads to the surmise that the failure of chromosome pairing is a cause of hybrid sterility.

It has been shown above (pp. 252-255) that individuals heterozygous for translocations produce spores or gametes some of which contain certain genes in excess and are deficient for certain other genes. In plants, spores with such duplications and deficiencies usually abort. In animals, gametes with abnormal complements of genes function, but the zygotes which they form are aborted. In either case, translocation heterozygotes are semisterile. Since translocations occur in the evolution of species, species hybrids are often translocation heterozygotes. This probably accounts for the sterility of some hybrids. Furthermore, we know that chromosomes are changed in evolution by inversions and other chromosomal aberrations. Chromosomes of different species may, then, contain the same gene loci but may have them arranged in quite different orders. When such chromosomes meet in the cells of an interspecific hybrid, they may be unable to accomplish meiotic pairing for purely mechanical reasons. Indeed, meiotic pairing depends upon attraction between homologous genes rather than between chromosomes as wholes. Chromosomes which contain genes differently arranged will hence be subjected at meiosis to conflicting attraction forces. Failure of the meiotic pairing and failure to establish chiasmata between homologous chromosomes interfere with the normal course of meiosis and may tend to make the hybrid sterile. Hybrid sterility produced by differences in the gene arrangements in the chromosomes of the parental species is termed *chromosomal sterility*.

The sterility of some hybrids cannot, however, be ascribed to difficulties in the meiotic pairing alone. For example, in the testes of male mules the degeneration of the prospective sex cell begins before the stage at which the meiotic pairing of chromosomes would normally take place. Conversely, in hybrids between some strains of *Drosophila pseudoobscura* and *D. persimilis* chromosomes seem to pair normally, but degenerative changes set in in spite of this. *Drosophila melanogaster* and *D. simulans* produce completely sterile hybrids, although their gene arrangements are more similar than among some races within a species which produce quite fertile hybrids. This suggests that the sterility of these hybrids is not caused by differences in gene arrangement between the parental species. An alternative explanation of the sterility is that the parents contribute genes

which interact in the hybrid in such a manner as to disturb the normal course of meiosis and of sex-cell formation. Hybrid sterility caused by the genic constitution of the hybrids rather than by differences in the gene arrangements in the parental chromosomes is called *genic sterility*. A good example is found in the amphidiploid hybrid of *Nicotiana sylvestris* \times *N. tomentosa*, which showed female sterility. Greenleaf found that this was due to the complementary action of genes from the parent species in causing abortion of embryosacs. It is, of course, quite possible that the sterility of some hybrids is caused by a combination of chromosomal and genic sterility. One of the possible methods of testing whether the sterility of a given hybrid is chromosomal or genic is examination of the behavior of the chromosomes in individuals in which the chromosomal complement has been doubled by polyploidy.

Autopolyploids. Chromosome division followed by failure of division of the nucleus and of the cell results in the production of cells which have every chromosome present in the original cell in duplicate and therefore have twice as many chromosomes as the ancestral cells do. Formation of such polyploid cells occurs spontaneously, and it may be induced artificially. Among polyploids, *tetraploids* have the diploid chromosome complement doubled, *triploids* have each chromosome in triplicate, and *pentaploids*, *hexaploids*, and *octoploids* have each chromosome represented five, six, or eight times. Polyploids that arise by doubling of the chromosomes of a strain are called *autopolyploids*, while those which come from doubling the chromosome complement of an interspecific hybrid are *allopolyploids*, or *amphidiploids*. There is no sharp dividing line between autopolyploids and allopolyploids, since polyploids derived from race hybrids or from hybrids between incipient species have intermediate properties.

An especially simple and effective method of inducing polyploids has been developed through use of the alkaloid colchicine. Weak solutions of this substance are applied to buds by immersion, by spraying, or in agar or lanolin or to seeds by soaking. In treated material a high proportion of dividing cells fail to carry division through to completion, the chromosomes dividing but the new cell wall failing to appear. Such cells are thus tetraploid and often give rise to pure tetraploid branches. Polyploids of higher order may be produced in the same way. This treatment has proved effective in a wide variety of plants and with the eggs of certain animals.

Autotetraploids usually (though not invariably) differ from their ancestral diploids in a number of characters, especially greater stature of stem and size of leaves and flowers ("gigas" type), these being due to the increased size of their cells. Other structural differences involve the shape of various organs, as of the leaves and capsule of *Datura* and the fruits of cucurbits. Autotetraploids are often phenotypically different from diploids in less concentrated cell sap, slower growth, and greater hardness.

The reproduction of autotetraploids may be almost normal. The tetraploid *Datura* produces viable gametes with $2n$ chromosomes (24) and a few with irregular numbers. These regular gametes result from the reduction of the 48 chromosomes by the formation of 12 groups of 4 chromosomes each (quadrivalents) and the passage of two homologues to each gamete. This is apparently a random process as shown by the segregation of two genes in tetraploid *Datura*. A purple-flowered tetraploid ($PP PP$) crossed with a white-flowered tetraploid ($pp pp$) gives purple in F_1 and an F_2 ratio of 35 purple:1 white, from which it has been inferred that the gametes formed by the F_1 ($PP pp$) were 1 PP :4 Pp :1 pp , the result expected from random assortment.

Although the fertility of autotetraploids is generally somewhat reduced by the formation of gametes with abnormal chromosome numbers, some may reproduce normally enough to become established as new types. But a single tetraploid arising in a dioecious plant or in bisexual animals would have little chance of perpetuating its kind since its diploid gametes would meet in general only the haploid gametes of the normal population and produce not tetraploids but a new $3n$, or triploid, type. Müntzing has reviewed the literature on autoployploids, and further details will be found in his paper.

Allopolyploids. A classical example of an allotetraploid is *Raphanobrassica* obtained by Karpechenko from hybrids between radish, *Raphanus sativus* (the diploid chromosome number, $2n = 18$), and cabbage, *Brassica oleracea* ($2n = 18$). Radish and cabbage cross with difficulty. The F_1 hybrids have 18 chromosomes, 9 of them contributed by the radish and 9 by the cabbage parent (Fig. 157).

At meiosis, the radish and cabbage chromosomes in the hybrid mostly fail to pair, the meiotic divisions are highly abnormal, and the spores usually degenerate, making the hybrid very nearly sterile—a typical case of sterility of an interspecific hybrid. However, in some cells the chromosome complement undergoes a doubling, and this leads to formation of a few seeds, from which some second-generation hybrids can be obtained. Most of these have 36 chromosomes, the sum of the chromosome numbers of the two parent species (9 pairs of radish and 9 pairs of cabbage chromosomes). Such tetraploid hybrids are remarkable for their giant size and even more so for their almost complete fertility and true breeding; since their morphological characters are intermediate between radish and cabbage, while they are infertile with both parent species, the name *Raphanobrassica* has been given to them (Fig. 157).

The chromosome behavior at meiosis in diploid *Raphanobrassica* is entirely normal. The 36 chromosomes which the plant has form 18 bivalents, and the embryo sacs and pollen grains carry 18 chromosomes, 9 of them representing the full radish complement and the other 9 the full cab-

bage complement. Fertilization results in new plants with 36 chromosomes and in no segregation of characters of the parental species.

The contrast between the normal chromosome behavior at meiosis in the diploid radish \times cabbage hybrid and the normal meiosis in the tetraploid *Raphanobrassica* is striking. No less striking is the contrast between the

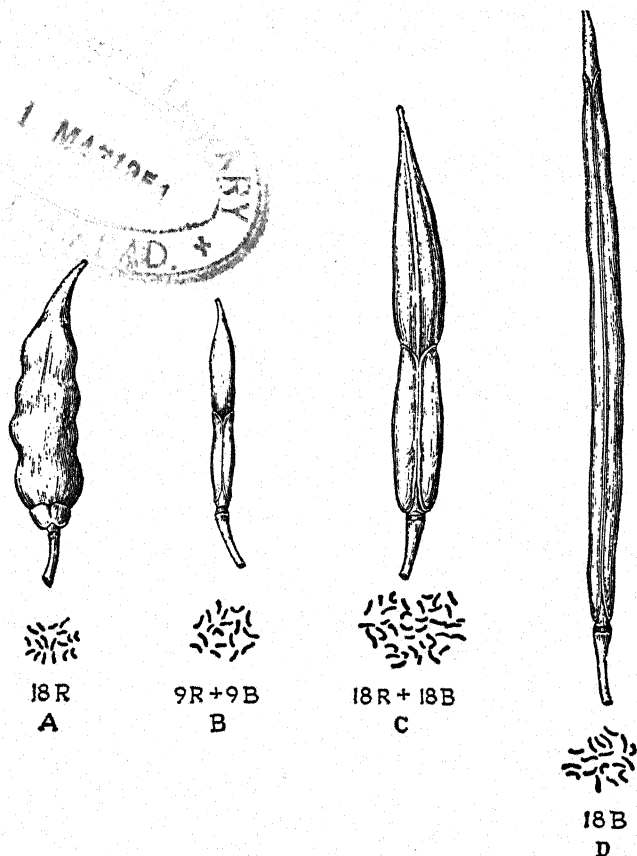


FIG. 157. Pods and somatic chromosomes (*R*, *Raphanus*; *B*, *Brassica*), of radish (*Raphanus*) *A*, cabbage (*Brassica*) *D*, their diploid hybrid *B*, and their allotetraploid hybrid *C*. (After Karpechenko.)

sterility of the diploid and the fertility of the tetraploid hybrid. The failure of bivalent formation in the diploid hybrids is here caused by dissimilarities in the gene arrangements in the radish and cabbage chromosomes. This is a case of *chromosomal sterility*. At meiosis radish chromosomes do not find normal mates among the cabbage chromosomes, and vice versa.

On the other hand, the tetraploid carries every radish and every cabbage chromosome in duplicate, and consequently every chromosome has a mate with a precisely similar gene arrangement, as the formation of the 18 bivalents clearly shows.

Allotetraploid hybrids are, however, by no means always fertile, nor do they always have normal meiosis. For example, tetraploid cells are frequently formed in the testes of the sterile male hybrids between *Drosophila pseudoobscura* and *D. persimilis*, but meiosis in such cells is just as abnormal as in the diploid ones, and no functional sex cells are formed. The sterility of these hybrids is evidently genic, caused by their genetic constitution. The tetraploid cells in the hybrids carry, then, the same genes as the diploid ones, except that every gene is reduplicated in the tetraploid. Abnormalities in meiosis accordingly persist, and fertility is not restored. The behavior of the allotetraploids furnishes, consequently, a method whereby chromosomal and genic sterility may be distinguished.

Polyploidy as a Method of Origin of Species. The tetraploid *Raphanobrassica* is not merely fertile and true-breeding, but it is to a considerable extent isolated reproductively from its progenitors, radish and cabbage. Crosses between *Raphanobrassica* and radish or cabbage succeed with some difficulty, and when they do, the progeny consists of triploid hybrids having 27 chromosomes. Of these, 9 are radish and 18 are cabbage chromosomes, or vice versa, depending upon whether *Raphanobrassica* is backcrossed to radish or to cabbage. At meiosis, 9 bivalents are formed, and 9 chromosomes are left without mates and remain univalent. The bivalents result evidently from pairing among the two sets of 9 chromosomes derived from one species, and the univalents are the chromosomes of the other species. At the meiotic divisions, the bivalents divide normally, sending a set of 9 chromosomes to each pole of the division spindle; but the univalents are distributed at random to the daughter cells so that the spores have varying chromosome numbers, from 9 to 18. Most of these spores degenerate, and the triploid hybrids are largely sterile.

Raphanobrassica is, then, effectively a new species produced in an experiment. It is important that species formation by allopolyploidy, by a method exemplified by *Raphanobrassica*, has taken place in nature in many plant genera. Furthermore, in at least one case an existing polyploid species has been resynthesized experimentally. Müntzing crossed two species of the mint family, *Galeopsis pubescens* and *G. speciosa*, both having 16 chromosomes in diploid condition. From the diploid hybrid, with 8 *G. pubescens* and 8 *G. speciosa* chromosomes, Müntzing eventually obtained a tetraploid with 32 chromosomes. This tetraploid proved to resemble morphologically a third species, also found in nature and known as *G. Tetrahit*, which likewise has 32 chromosomes in body cells and has 16

bivalents at meiosis. It is probable that *G. Tetrahit* arose in nature as an allotetraploid hybrid of *G. pubescens* and *G. speciosa* or species very similar to them. The "artificial *Tetrahit*" obtained in the experiment has been crossed to the natural *G. Tetrahit*. The cross succeeds easily, and the hybrid resembles both parents, is fertile, and forms 16 bivalents at meiosis.

Less complete but still convincing evidence of the origin of species by polyploidy is available for several other plant species, including some important cultivated plants. Thus, commercial tobacco, *Nicotiana tabacum*, with its 24 pairs of chromosomes is probably an amphidiploid, comprising genomes derived from *N. sylvestris* ancestry and from *N. otophora* or the closely related *N. tomentosa*. When each of the monosomic (23 chromosomes) types of *tabacum* is crossed with *N. sylvestris*, it is possible to classify by pairing behavior each of the monosomic hybrids as monosomic for a member of the *tomentosa* or of the *sylvestris* genome. Twelve monosomics fell into the first and 12 into the latter category; hence the assumed amphidiploid nature of *tabacum* is confirmed. These facts have been recently reviewed by Goodspeed.

The American cultivated cottons, *Gossypium barbadense* and *G. hirsutum*, have 26 pairs of chromosomes and represent amphidiploid derivatives from crosses of species with 13 pairs. A group of diploid species with 13 pairs occurs in the wild state in Central America, Peru, and the Galapagos Islands; another group occurs in the Old World (tropical Asia, Africa, and Australia). The American tetraploid cottons contain 13 pairs of chromosomes similar to those found in the American diploids and 13 pairs similar to those in the Old World diploids. The chromosomes of the Old World diploids do not resemble those of the American diploids. The tetraploid species probably arose from hybrids between the American and the Old World diploid species, by doubling of the chromosomal complement. An intriguing but unsolved problem is just where and when the American and the Old World diploid species met and crossed. Their present geographic distributions are separated by thousands of miles of tropical oceans, and wild cottons are so definitely tropical and subtropical plants that it is difficult to conceive any of them migrating overland, through the arctic territories in the region of Bering Strait, which seems to furnish the only possible land connection between America and Asia. As a solution of this difficulty, Harland proposed the hypothesis that the formation of the tetraploid cotton species took place very long ago, in early Tertiary or even in Cretaceous times, when the climate of the world was much warmer than it is now. Conversely, Hutchison, Silow, and Stephens suppose that seeds of Old World diploid cottons were transported from Asia across the Pacific Ocean to the west coast of South America by Polynesian mariners. These introduced species then crossed spontaneously with the native American

diploids, probably with *Gossypium Raymondii* growing in wild state on the coast of Peru. Doubling of the chromosome complement then gave rise, only some centuries ago, to the American tetraploid cottons.

Species of wheat fall into three groups: diploid einkorn wheats with 14 chromosomes (7 pairs), tetraploid emmer, or hard, wheats with 28 chromosomes (14 pairs), and hexaploid *vulgare*, or soft, wheats with 42 chromosomes (21 pairs) (Fig. 158). The work of Sax, Kihara, and others showed that the chromosome complement of emmer wheats contains a set of 7 chromosomes similar to those of einkorn wheat and a set of 7 other chromosomes derived from some other plant. *Vulgare* wheats contain 14 pairs of chromosomes similar to the emmers and 7 pairs similar to those found in some species of the grass genus *Aegilops*. The emmer wheats must, then, have arisen as allotetraploid hybrids. The *vulgare* wheats are allohexaploid hybrids derived from emmerlike and *Aegilops*like ancestors.

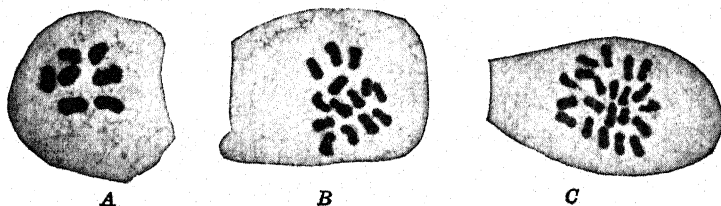


FIG. 158. Chromosome differences in species of wheat. Reduction division in pollen mother cells of A, *Triticum monococcum*, 7 chromosomes; B, *T. durum*, 14 chromosomes; C, *T. vulgare*, 21 chromosomes. (From Sax.)

Animal Polyploids. The facts presented on the foregoing pages show that two fundamentally distinct methods of species formation may be distinguished. In probably all groups of organisms that reproduce sexually and by cross-fertilization, species arise by gradual accumulation of genetic differences and divergence of geographic races. On the other hand, in many plant genera species arise through polyploidy, chiefly through allopolyploidy. By comparison with the slow emergence of species from races, species formation by polyploidy may be said to be an instantaneous process. This instantaneous method of species formation occurs but rarely in the animal kingdom. Chromosome numbers in species of many plant genera and families form series of multiples of a basic number, thus giving an indirect evidence of the occurrence of polyploidy in evolution. Such multiples are rare among animals. Although orders and classes of animals may have rather different chromosome numbers (for example, marsupials have 6 to 13 chromosome pairs, while placental mammals have 7 to 39 pairs), there is no good reason to think that such differences could not have evolved by translocation (see p. 261) rather than polyploidy.

Spontaneous chromosome doubling leading to formation of polyploid

cells and polyploid individuals is not rare in animals, as shown particularly by Fankhauser and his students in salamanders (Fig. 188, p. 427). Yet polyploid races and species have been found mainly in the aberrant groups which multiply by parthenogenesis rather than by cross-fertilization. Such is the case in the shrimp *Artemia* according to Artom, the sow bug *Trichoniscus* according to Vandel, and in the moth *Solenobia*, according to Seiler.

Among the species of weevils of the family Curculionidae, Suomalainen has found that all bisexual species have the same chromosome number ($2n = 22$) and sex determination of the *Drosophila* type (XY males). All of the related species which are parthenogenetic are polyploid with 33, 44,

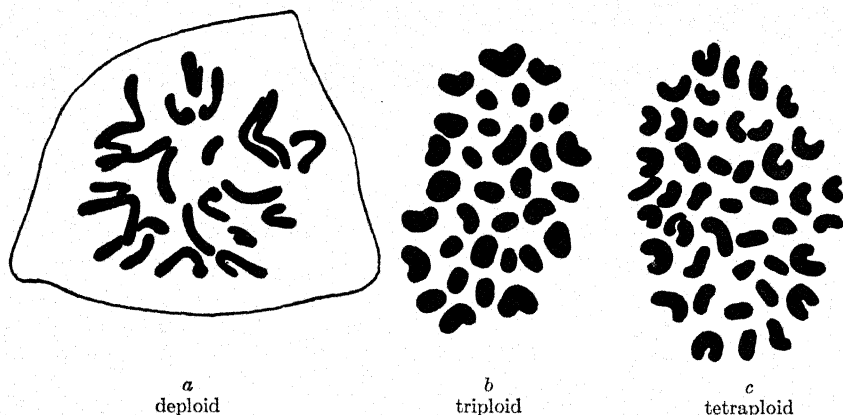


FIG. 159. *a*, spermatogonial metaphase in the beetle, *Otiiorhynchus arcticus*; *b*, meiotic metaphase (equatorial) of *O. ovatus*; *c*, meiotic metaphase of *O. dubius*. (After Suomalainen.)

or 55 chromosomes. The establishment of bisexual polyploid races in natural populations of an animal species with male heterogamety would of course encounter difficulties because of the appearance of sterile intersexes among the triploids as in *Drosophila*. This would tend to restrict the origin of polyploid races to species with facultative parthenogenesis, and this would be rather rare.

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CHAPTER XV

THE DETERMINATION OF SEX

The essential feature of sexual reproduction is the union of two different gametes. When these gametes come from individuals with different heredities, sexual union provides the opportunity for a greater hereditary variability than could possibly arise under asexual reproduction. The segregation and recombination of genes which occur under bisexual reproduction produce a great variety of genotypes, some of which are less well adapted than others in various environments, and these are likely to be eliminated by natural selection, while the better adapted ones will be preserved and increased. In contrast with sexual species, the evolutionary plasticity of species which reproduce asexually is greatly restricted. In a clone, adaptively valuable combinations of genes can be formed only in the relatively improbable case that all the genes concerned undergo the proper mutational changes in the same line of descent. Thus, the major advances of evolution became possible only after sexual reproduction had evolved.

It is not surprising, then, that sexual union is the predominant or only method of reproduction in all higher animals and the usual method in plants and in many lower organisms. The fusion of nuclei of different origin, followed by segregation and recombination of genes, occurs in all sexual reproduction, but this essential function is accomplished in many different ways in different animals and plants.

In some lower organisms the gametes that unite are at least superficially similar, although they may be physiologically different; but in all others, male and female gametes are easily distinguishable, the eggs being much larger and less motile than the sperms. In hermaphroditic, or monoecious, forms, male and female gametes are produced by the same individual, while in dioecious, or bisexual, organisms, one individual normally produces only eggs or only sperms. This primary difference between male and female individuals is usually accompanied by more or less profound distinctions between the sexes. In animals these are often very marked in the anatomical and external features involved in courtship, mating, fertilization, and care of offspring; and in the higher animals these differences are reinforced by physiological differences, such as those in the endocrine systems associated with ovary and testes, which accentuate the external differences in secondary sexual characters. In plants, where the haploid

gametophyte generation is either male or female, while the sporophyte generation which alternates with it is nonsexual and bears spores, the distinction between male and female is less sharply marked than in animals. In most seed plants both male and female gametophytes are borne on the same plant (sporophyte) or even in the same hermaphroditic flower. In some species, however, male and female gametophytes are borne on different plants, which are thus *dioecious*. Sexual distinctions between male and female plants, however, seldom extend beyond the character of the floral parts.

The Chromosome Theory of Sex Determination. A problem of fundamental importance is how to explain the occurrence in many species of two different types of individuals: males and females. The sexual differences are, in general, sharp and discontinuous; they indicate profound differences between members of the same species. What determines the fact that one of these individuals will be a male and produce sperms, while another develops as a female and produces eggs? These two kinds of individuals among the higher animals are usually produced in approximately equal numbers and fall into distinct alternative classes. These facts suggest the operation of some exact mechanism in inheritance. The problem of finding the mechanism by which this primary difference between the sexes originates we shall call the problem of *sex determination*.

Literally hundreds of hypotheses had been proposed in the centuries preceding 1900 in vain attempts to solve the riddle of sex determination, but these were all rendered obsolete and unnecessary by some observations of geneticists and cytologists in the years immediately after 1900. Two of these discoveries which are of prime importance for the problem of sex determination have already been described (pp. 175-191). One of these was the disclosure of a difference in chromosome constitution between male and female animals (Fig. 160) and this was later extended to several species of dioecious plants. The other was the proof that sex-linked genes are located in the sex chromosome and that they thus identify the offspring receiving this chromosome so that its distribution to the gametes and progeny may be inferred from genetic evidence. These discoveries showed that the primary sexual difference is inherited like other Mendelian traits and brought the problem definitely into the field of genetics.

It was found that in bisexual or dioecious species the genetic sex of the individual was usually decided at fertilization by the sex-chromosome constitution of the uniting gametes. This happens in different ways in different species, as shown in Table XL.

The chromosome theory of sex determination was proved by the correlation between the distribution in inheritance of sex chromosomes and of the sex-linked genes.

The crucial experiments were those of Bridges in 1916 on nondisjunction of sex chromosomes in *Drosophila*. These showed that the chromosome complement of an individual may be predicted from a knowledge of its

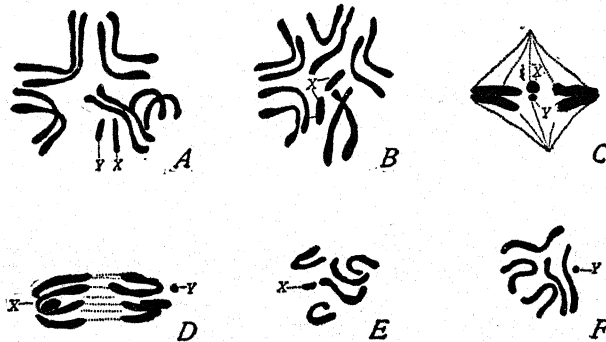


FIG. 160. The chromosomes of a fly (*Calliphora*) showing X and Y chromosomes in A, spermatogonia; B, oögonia; C and D, first spermatocyte (reduction) division; E and F, X and Y spermatocytes after reduction. (From *The Cell in Development and Heredity*, by E. B. Wilson, 8d ed., copyright 1925 by the Macmillan Company. Reprinted by permission.)

pedigree; and, conversely, by knowing the chromosomal constitution one may predict the course which certain sex-linked traits will follow in inheritance. The proof by L. V. Morgan (p. 189) that attached X chromosomes with their genes go from mother to daughter, that is, that 2 X chromosomes added to a set of autosomes produce a female even when both

TABLE XL. VARIETIES OF SEX DETERMINATION

(A = set of autosomes; X = sex chromosome)

Type	Gametes		Zygotes	
	Sperm	Eggs	Males	Females
Protenor (XO ♂).....	$\frac{1}{2}$ A + X; $\frac{1}{2}$ A + O	all A + X	2 A + X	2 A + XX
<i>Drosophila</i> (XY ♂).....	$\frac{1}{2}$ A + X; $\frac{1}{2}$ A + Y	all A + X	2 A + XY	2 A + XX
Bird and moth (XY ♀)....	all A + X;	$\frac{1}{2}$ A + X; $\frac{1}{2}$ A + Y	2 A + XX	2 A + XY
<i>Fumea</i> (XO ♀).....	all A + X	$\frac{1}{2}$ A + X; $\frac{1}{2}$ A + O	2 A + XX	2 A + X

X's come from the mother, added further confirmation to the theory. The analysis made by T. H. Morgan and C. B. Bridges (1919) of gynandromorphs in *Drosophila* may perhaps be regarded as having furnished the final proof.

Gynandromorphs. Exceptional individuals of normally bisexual species, which are male on one side and female on the other or have some parts of the body male and other parts female, have been recorded in biological literature in various organisms. Such sexual mosaics, or *gynandromorphs*, occur from time to time in *Drosophila* (Fig. 161). Bridges and Morgan found, however, that when gynandromorphs appear in crosses involving sex-linked genes the distribution of the sex-linked traits is often different in the female and male tissues of the same individual. In most cases their distribution may be explained if one supposes first that the gynandro-

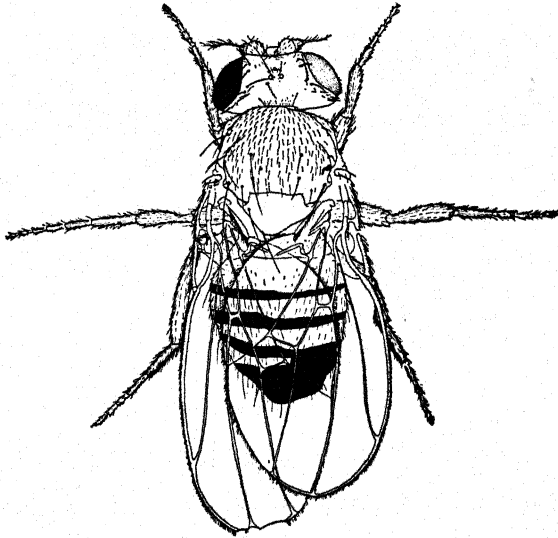


FIG. 161. A gynandromorph in *Drosophila melanogaster*. The left side of the body is predominantly female and the right side, male. (After Bridges.)

morphs arise from zygotes that initially have two X chromosomes and hence should develop into females and second that one of these two X chromosomes is lost during the cleavage of the fertilized egg in some nuclei, so that the tissues which are formed by the descendants of these nuclei have only one X chromosome (and no Y chromosome) and are therefore male. For example, the cross of a wild-type *Drosophila* female to a male with the sex-linked recessive gene yellow normally produces wild-type daughters (heterozygous for yellow) and wild-type sons. If gynandromorphs appear in the progeny of this cross, the female parts of the body are always wild type and never yellow, while the male parts may be either wild type or yellow. The explanation is clear: the female body parts in the gynandromorphs have two X chromosomes, one of which carries the wild-type

allele of yellow; the male parts on the other hand may be either wild type or yellow depending upon whether the X chromosome which is eliminated carries yellow or the wild-type allele of this gene. If a gynandromorph has female sex organs, it is often fertile as a female, but gynandromorphs with male sex organs are invariably sterile. This is as should be expected, because the male parts of gynandromorphs have no Y chromosome and *Drosophila* males lacking the Y chromosome are known to be sterile (see p. 190 and Fig. 161).

In silkworms gynandromorphism has appeared as an inherited condition which segregates as though due to a single gene, which also determines



FIG. 162. Mosaic silkworm larvae. (From Goldschmidt and Katsuki.)

the production of somatic mosaics (Fig. 162). The gynanders arise from eggs with *two* nuclei, one XX from which the male parts descend; the other XY, which gives rise to the female parts. Somatic mosaics result when one nucleus is, for example, *Aa*, and the other, *aa*. These two diploid nuclei arise from the retention of one polar-body nucleus and fertilization of both this and the egg nucleus by different sperms; and this peculiarity, determined by the action of one gene, may result in individuals mosaic for sexual or somatic characters or both.

Intersexes and Supersexes in *Drosophila*. We have seen that by about 1920 the genetic and cytological work on *Drosophila* had established that sex in *Drosophila* is determined by X chromosomes, two X's giving rise to a female and one X to a male. The Y chromosome is of little importance

in sex determination: individuals with two X's and a Y are normal females (XXY females, see Chap. VIII), and individuals with one X and no Y are morphologically normal, although sterile, males (XO males).

In 1922 Bridges published his classical work on triploid intersexes and supersexes in *Drosophila melanogaster*, in which he showed that the above simple theory, although correct as far as it goes, is incomplete. He found some females which proved to have every chromosome, including the X chromosome, in triplicate. Such *triploid females* resemble the normal diploid females, except for roughened eyes and a few other morphological

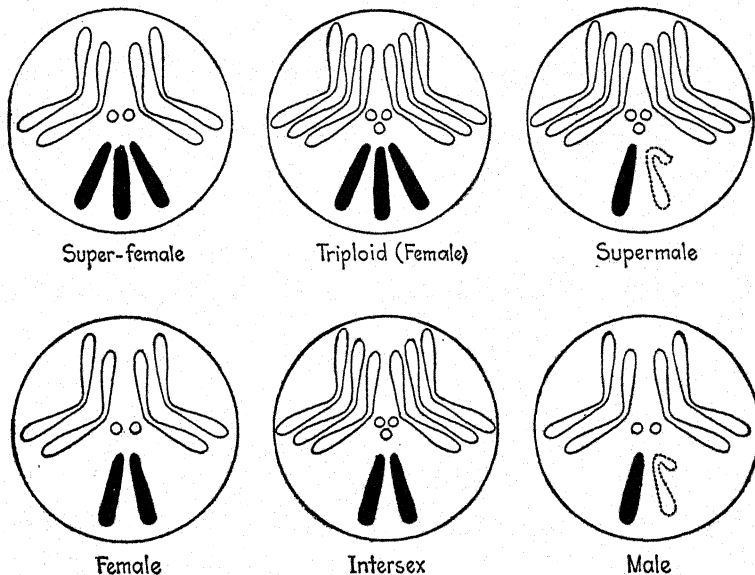


FIG. 163. Effect on sex of the balance between X chromosomes (solid) and autosomes (outlined) in *Drosophila melanogaster*.

details; and they are fertile.* Crossed to normal diploid males, the triploid females produce an array of eight sex types in the offspring, as follows: (1) triploid females, with 3 X chromosomes and 3 sets of autosomes (a "set" of autosomes consists of a second and a third chromosome; the small fourth chromosome may be present in single dose or in duplicate); (2) normal diploid females, with 2 X's and 2 sets of autosomes; (3) diploid XXY females with 2 X's, 1 Y, and 2 sets of autosomes; (4) intersexes with 2 X chromosomes and 3 sets of autosomes; (5) intersexes not distinguishable from the foregoing class except in having 2 X chromosomes, 1 Y chromosome, and 3 sets of autosomes; (6) normal males with 1 X, 1 Y, and 2 sets of autosomes; (7) superfemales with 3 X's and 2 sets of autosomes; (8) supermales with 1 X, 1 Y and 3 sets of autosomes (Fig. 163).

Intersexes are sterile individuals intermediate between females and males; superfemales and supermales are also sterile individuals showing certain minor morphological differences from females and males, respectively. Bridges has interpreted his observations as follows:

The intersexes and $3n$ (triploid) types lead to the conclusion that sex in *Drosophila melanogaster* is determined by the autosomes as well as by the X chromosomes, the ratio of autosomes to X's being the significant relation. The old formulation of $2X = \text{♀}$ is at once seen to be inadequate; for here we have individuals with two X chromosomes and yet [they] are not females. They are shifted out of the female class by the presence of an extra set of autosomes, and thereby the autosomes are proved to play a positive role in the production of sex. Since the intersexes differ from females by the assumption of certain male characters, this effect of the auto-

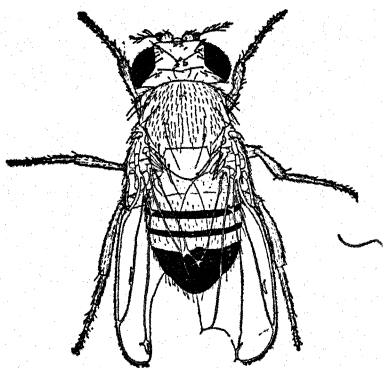


FIG. 164.

Fig. 164. Male-type intersex ($2X:3A$) in *Drosophila melanogaster*. (From Bridges.)

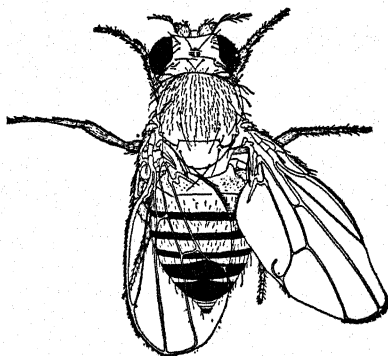


FIG. 165.

Fig. 165. Female-type intersex ($2X:3A$) in *Drosophila melanogaster*. (From Bridges.)

somes is due to an internal preponderance of "male-tendency" genes. We may now reformulate the sex relation as follows: Both sexes are due to the simultaneous action of two opposed sets of genes, one set tending to produce the characters called female and the other to produce the characters called male. These two sets of genes are not equally effective, for in the (chromosome) complement as a whole the female-tendency genes outweigh the male and the diploid (or triploid) form is female. The male-tendency genes in the autosomes are more numerous or more effective than those in the X chromosome, while the net effect of genes in the X chromosome is a tendency to the production of female characters. When in a diploid zygote the relative effectiveness of the female-tendency genes is lowered by the absence of one X, the male-tendency genes outweigh the female, and the result is the normal male. When the two sets of genes are acting in a ratio between these two extremes, as in the case in the ratio of $2X:3$ sets of autosomes ($2X:3A$), the result is a sex intermediate—the intersex.

Sex is, consequently, determined by the genic balance, that is, by the preponderance of the male-tendency or the female-tendency genes. The

genic balance is, in turn, governed by the ratio of the number of X chromosomes to the number of sets of autosomes in the zygote at fertilization. As shown in Table XLI, this ratio is 1.0 in any individual which is to develop into a female but only .5 in a male. If the ratio is intermediate between 1.0 and .5, the resulting individual is neither a female nor a male but an intermediate, an intersex (.67). Superfemales have a ratio of 1.5, or higher than that in normal females, and supermales .33, which is lower than in normal males.

TABLE XLI. SEXUAL TYPES IN *Drosophila melanogaster* (After Bridges)

Sex	X chromo- somes	Sets of autosomes (A)	Sex index (ratio X/A)
Superfemale.....	3	2	1.5
{ tetraploid.....	4	4	1.0
Normal female { triploid.....	3	3	1.0
{ diploid.....	2	2	1.0
{ haploid*.....	1	1	1.0
Intersex.....	2	3	0.67
Normal male.....	1	2	0.50
Supermale.....	1	3	0.33

* A whole individual of this type has not yet been obtained, but patches of tissue with one X and one of each autosome have been found in diploid flies. These patches show female characters.

Influence of Environment on Intersexes in *Drosophila*. Bridges' theory left a rather striking fact unexplained. The triploid intersexes all have two X chromosomes and three sets of autosomes. Yet the sexual characters of intersexes vary all the way from a condition resembling normal males (male-type intersexes) through various intermediate situations to a condition approaching normal females (female-type intersexes). If sex is determined by the ratio of X chromosomes to autosomes, what is the origin of this wide variation among the intersexes?

Triploid females were allowed to produce offspring at different temperatures, and the sexual characteristics of the intersexes which appeared in their offspring were compared. The average sex type in the intersexes which developed at high temperatures was shifted in the female direction and in those grown at low temperatures was altered in the male direction. Consequently high temperature has a feminizing and low temperature a masculinizing effect in *Drosophila*.

Influence of Genetic Modifiers on Intersexes in *Drosophila*. Some triploid females produce intersexes that are on the average more feminine or more masculine than others. By selecting females that produce the most feminine or most masculine intersexes in several generations, it is possible to obtain triploid strains which produce only female-type or only

male-type intersexes at a given temperature and under certain environmental conditions. These intersexes evidently have the same chromosome complements, but they differ in that some of them have more feminizing and others more masculinizing modifying genes.

The female-determining effect of the X chromosome and the male-determining effect of the autosomes may be explained in one of two ways. First, the X chromosome may contain a single gene or a group of closely linked genes which determines femaleness (the "female sex differentiator"), and one of the autosomes may carry a gene or a small section which determines maleness (the "male sex differentiator"). Second, many or even all the genes in all parts of the X chromosome may be female determining, and many or all the genes in the autosomes may be male determining. Any one of these genes taken separately may have a small effect on sex, but their aggregate effect may be powerful.

To discriminate between these possibilities, triploid females were outcrossed to diploid males which carried duplications for some sections of the X chromosome or of other chromosomes. In the progeny of these crosses, intersexes were obtained which carried two X chromosomes and three sets of autosomes and also intersexes which had, in addition, a fragment of a third X chromosome or autosomal fragment. Now, if a given section of the chromosome represented in the fragment contains genes that modify the development toward femaleness, the intersexes which carry the duplicating fragment should be more femalelike than the intersexes without the duplication. Conversely, if the duplication contains genes that tend toward maleness, the duplication-carrying intersexes should be more malelike than those without the duplication. Just which parts of a chromosome are contained in a duplication can be determined genetically as well as cytologically, as explained in Chapter X. The experiments showed that intersexes which carry duplications for any section of the X chromosome (except for the heterochromatic part which includes the centromere, see p. 264) are always more femalelike than intersexes without duplications. The longer the duplication—in other words, the more female-tendency genes are added to an intersex—the more femalelike it becomes. Intersexes which carry duplications for about one-third of the euchromatic part of the X chromosome (that is, carrying about $2\frac{1}{3}$ X chromosomes) not only resemble females morphologically but deposit fertile eggs and are, therefore, functional females. On the other hand, no gene and no short section of the X chromosome by itself transform intersexes into females. It can be concluded that the X chromosome of *Drosophila melanogaster* has no single female sex differentiator but that it contains many female-determining genes scattered throughout its euchromatic length, the combined effects of which make the chromosome female determining. The situa-

tion of the autosomes is as yet not clear, since at least some duplications for sections of the autosomes are neither male nor female determining.

Factor of Safety. We have seen that the sexual state of triploid intersexes in *Drosophila* can easily be altered by external influences such as temperature, as well as by relatively slight internal changes, such as modifying genes or duplications for short sections of the X chromosome. The remarkable and significant fact is that the same influences have no noticeable effects on the sex of normal males and females. Females and males grown at temperatures as high and as low as *Drosophila* can withstand show no trace of intersexuality. Neither is there evidence of intersexuality in females and males which contain the same modifying genes or the same duplications, which strongly alter the sexual traits of intersexes. The sexual balance of males and females is evidently buffered against disturbance by environmental and genetic modifiers. The sexual balance of intersexes is, on the contrary, so poised that relatively slight upsets engender considerable alteration in sexual characters.

It should be kept in mind that normal functioning of the sex mechanism is obviously important for the preservation of the species. Natural selection eliminates all variants in which this mechanism is easily upset by environmental agencies (temperature) or by genetic modifiers that occur in the species. Conversely, genetic variants that add a "factor of safety" to the sexual balance of the normal females and males, by making the femaleness of females and the maleness of males so strong that it cannot be easily upset, are favored by natural selection and therefore become established in the process of evolution as the normal condition of the species.

The sexual phenotype, as any other phenotypic trait, is determined by the interaction of the genotype with the environment (Chap. I). But the range of reaction of the genotype of a species is so adjusted in the process of evolution that the phenotypes engendered in the environment in which the species normally lives possess sufficient fitness to perpetuate themselves. The ranges of reaction of females and males are set by the chromosomal mechanism in such a way that two X chromosomes always produce a normal female and one X a normal male. Triploid intersexes obtained in laboratory experiments are, on the other hand, not normal constituents of the species populations. Their sexual balance has no factor of safety, and natural selection tends to prevent the production of intersexes rather than to establish a definite intersexual phenotype.

Mutations nevertheless arise which upset the sexual balance sufficiently to transform females into intersexes. Such mutants, due to changes in a gene located in one of the chromosomes, have been described in *Drosophila simulans*, *D. virilis*, *D. pseudoobscura*, and *D. melanogaster*. These intersexes have, of course, the same chromosome complement as normal diploid

females: two X chromosomes and two sets of autosomes. Their intersexual condition is caused not by disturbance of the X-chromosome-autosome ratio but by a qualitative change of one of the chromosomes. Such intersexes are known as *diploid intersexes*.

Diploid Intersexes in the Gypsy Moth. Beginning about 1911, Goldschmidt published detailed studies of diploid intersexes in the gypsy moth, *Lymantria dispar*. This moth, remarkable for its striking *sexual dimorph-*

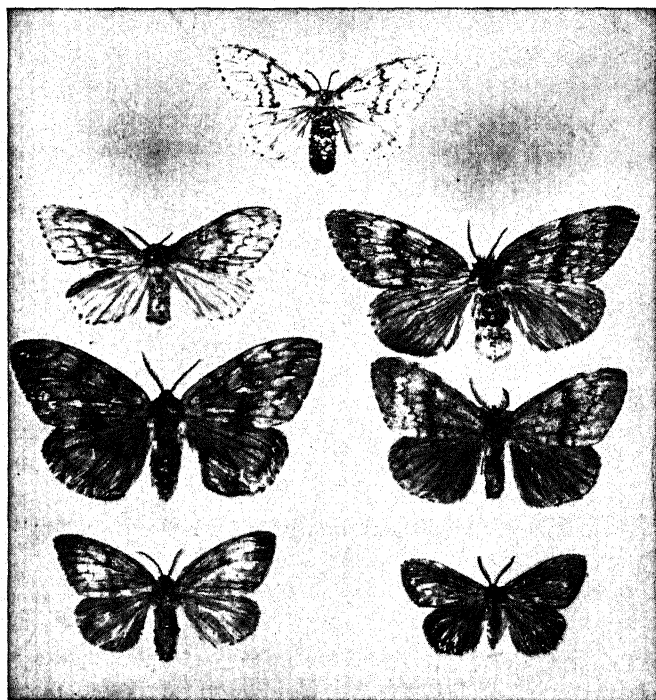


FIG. 166. Series of female intersexes in *Lymantria dispar*, from normal female (above) through increasing grades of maleness to sex-reversed male (lower right). (From Goldschmidt.)

ism (external differences between females and males), occurs naturally in Europe, northern Africa, and northern Asia. It has been introduced and has become a serious pest in New England. The offspring of matings between females and males from any one geographic locality consist of females and males only, but if strains of European origin are crossed with those from Japan or if strains from northern and southern Japan are intercrossed, the progenies contain some intersexes, as shown in Table XLII and Fig. 166.

To account for these results, Goldschmidt supposes that sex in the gypsy moth is determined by the interaction of factors for maleness carried in the X chromosome and factors for femaleness carried in the cytoplasm. These factors vary in "strength." In any one race the strengths of these factors are so adjusted by prolonged natural selection that two doses of maleness (carried in two X chromosomes) dominate the femaleness and produce a normal male and one dose of maleness (one X chromosome) is dominated by the femaleness and gives rise to a normal female. However, the sex factors are relatively "weak" in European and "strong" in Japanese strains. Accordingly, if a European female is crossed to a Japanese male, one Japanese X chromosome with its maleness is not entirely dominated by the relatively weak femaleness inherited from the European parent; the XY individuals that should develop into females become intersexes. In the

TABLE XLII. SEXUAL TYPES WHICH RESULT FROM CROSSES OF EUROPEAN AND JAPANESE STRAINS OF THE GYPSY MOTH

Cross	Parents	Sons	Daughters
1	European "weak" ♀ × Japanese "strong" ♂	Normal	Intersexual
2	Japanese "strong" ♀ × European "weak" ♂	Normal	Normal
3	F ₂ from cross 1	Normal	½ normal, ½ intersexual
4	F ₂ from cross 2	½ normal, ½ intersex	Normal

gypsy moth it is possible to distinguish intersexual females and intersexual males, that is, individuals which according to their chromosomal constitution (XY or XX) should be females or males, respectively, but which become intersexes on account of the unbalance of the sex-determining factors in the different races. The sex phenotype is not rigidly set by the chromosomes.

Goldschmidt has also shown that all intermediates between strong and weak sex factors can be found in different races. The "weakest" race of the gypsy moth lives on the northern island of Japan (Hokkaido) while the "strongest" race occurs at the northern extremity of the main Japanese island (Honshu). Crosses of these races result not in intersexuality but in sexual transformation (sex reversal); that is, some of the XY individuals develop into "transformed," or sex-reversed, males and XX individuals into "transformed" females. Thus, the sex phenotype may be the opposite of the one chromosomally established. As one proceeds southward

from the northern tip of Honshu, "strong" races are met with less and less, until in southern Japan and Korea the strength of the sex factors is not very different from that encountered in European populations.

Goldschmidt and his coworkers have also proposed a mechanism by which the sex factors influence the development of the sex phenotype. The essential assumption is that intersexes begin their development as females (or as males) and develop as such up to a certain critical point, the so-called "turning point," after which their development is of the opposite sexual type. The mixture of female and male traits found in intersexes is thus explained as due to the switching from the female developmental course to the male course, or vice versa, occurring earlier or later in development. A similar mechanism may operate also in the triploid intersexes in *Drosophila*, where the intersexes seem to start their development as males but complete it as females. The hypothesis of the turning point has, however, met with many objections and cannot be regarded as established.

Sex in *Bonellia*. The normal female and male sexual phenotypes are, like any other phenotypic traits, the outcome of developmental processes directed by genes and enacted in certain environments. The chromosomes which determine sex must be viewed as merely convenient switch mechanisms evolved by natural selection as adaptations to fulfill an important biological function, that of production of two stable sexual states. It is interesting that other switch mechanisms, which evidently fulfill the same function in a satisfactory manner, occur in nature. Those in *Bonellia* and in hymenopterous insects are most noteworthy.

Bonellia viridis is a marine echiuroid worm with an extreme sexual dimorphism. A female is about an inch long and possesses a fairly complex anatomical organization. Males are of the size of large infusoria and have rudimentary alimentary and other organs; they live as parasites in the uterus of the female. Baltzer and his collaborators have shown that the sex of a larva of *Bonellia* is determined by chemical stimuli emanating from the environment in which they live; and that larvae which settle on the proboscis of a female develop into males, while larvae which lead a free life develop into females. Intersexes can be obtained if larvae which have spent some time in contact with a proboscis are removed from it and forced to live away from other females. It appears that the proboscis of a female contains a substance of hormone type which exerts a powerful influence on the sexual traits of larvae.

Sex Determination in Hymenoptera. In ants, bees, and wasps parthenogenesis is widespread. Males have never been known in nature to develop from fertilized eggs. Females usually arise from fertilized eggs, but in some species alternation of generations occurs, a bisexual generation from unfertilized eggs alternating with a female generation from fertilized

eggs. In other species no males are known, and females produce females indefinitely by parthenogenesis. Females are always diploid, even though they develop parthenogenetically. Males are haploid, and all sperms from any one male are alike, no matter how mixed the race from which he comes.

In the parasitic wasp *Habrobracon juglandis*, Whiting has shown by means of many mutant genes that its heredity resembles that of the honey-bee. A recessive female crossed with a dominant male produces recessive males and dominant females; but in this case the males are from unfertilized eggs, and there is no constant sex ratio to be expected. An old mated female that has used up her supply of sperm or an unmated female produces only males.

If the parents are closely related, a few sons appear which, unlike their normal haploid brothers, have fathers, for they show the dominant traits of both parents. These biparental males have 20 chromosomes like their sisters instead of 10 like their haploid brothers. They are highly inviable, and fraternities in which they occur have very low egg hatchability.

Analysis of this situation by Whiting and his students led to an interpretation somewhat resembling the story of self-sterility alleles known in certain plants. Populations of *Habrobracon* contain a variety of sex alleles, which can be designated S^1 , S^2 , S^3 , etc. The haploid males are, then, of as many kinds as there are S alleles in the populations. A diploid zygote which contains two *different* alleles (S^1S^2 , S^1S^3 , S^2S^3 , etc.) develops into a normal female. But if both the father and the mother contain the same allele, some zygotes homozygous for a given sex allele (S^1S^1 , S^2S^2 , etc.) are formed and then develop into the poorly viable biparental males. Such homozygotes are formed as a result of inbreeding, and accordingly they occur more frequently in laboratory experiments than in nature.

Dreyfus and Breuer found that in another parasitic wasp, *Telenomus fariai*, inbreeding is a regular occurrence, because brothers and sisters copulate before they leave the shell of the host in which they develop. These authors have found in *Telenomus* a very special chromosomal mechanism which makes inbreeding compatible with a method of sex determination resembling that in *Habrobracon*. No biparental males are produced in *Telenomus*.

Sex Determination in Lower Algae. Sexual reproduction occurs in most groups of lower organisms. Bacteria and blue-green algae have for a long time been believed to be strictly asexual, but the work of Lederberg suggests that some kind of sexual process may occur in at least certain bacteria.

Hartmann and his school have made important studies on sex in lower algae. Strains of the unicellular alga *Chlamydomonas eugametos* can be

classified into two groups, arbitrarily designated plus and minus. Plus cells never unite with other plus, and minus never unite with minus, but plus cells under certain conditions fuse with minus and form diploid zygotes. The zygotes undergo two meiotic divisions and give rise to four flagellate haploid zoospores, two of which belong to the plus and the other two to the minus type. The zoospores may reproduce asexually for any number of generations; thus, plus and minus strains of *Eudorina elegans* were perpetuated asexually for twenty-three and fifteen years, respectively, which corresponds to thousands of division steps. They may, however, undergo another sexual fusion soon after their origin from the zygote.

In some varieties and species the individuals which fuse are visibly different, while in others the plus and minus strains are visibly so much alike that neither can be labeled female or male. Nevertheless, they are physiologically distinct. Moewus found that after exposure to blue or violet light the plus strains of *Chlamydomonas eugametos* var. *simplex* secrete in the culture fluid "sex substances," or *gamones*, which consist of a mixture of cis- and trans-isomers of crocetin dimethyl ester in a ratio approaching 75 parts of cis- and 25 parts of trans-isomer. Other varieties or related species produce the same gamones as indicated above but in different ratios. Thus, the plus strains of var. *synoica* give about 65 per cent of the cis- and 35 per cent of the trans-isomer, and the minus strains 35 per cent cis- and 65 per cent trans-. In var. *typica* the plus strains give about 85 per cent cis- and 15 per cent trans-isomer, in the minus strains the relations being again reversed. Finally in *C. Braunii* the plus strains give about 95 per cent cis- and 5 per cent trans-, and the minus strains give 5 per cent cis- and 95 per cent trans-isomer. Interestingly enough, if plus strains of var. *synoica* are placed with plus strains of var. *typica* or of *Braunii*, they evince a sufficient sexual reaction to each other so that sexual fusions occur. Similarly, minus strains of *synoica* undergo fusion with minus strains of *typica* or *Braunii*. This phenomenon, discovered also in forms other than *Chlamydomonas*, has been called relative sexuality. Sexual fusion may take place between strains that show a certain amount of difference in the relative concentration of the cis- and trans-isomers. Since the plus strains (sometimes designated as "females") of *Braunii* differ greatly from the plus strains of *synoica*, they behave with respect to each other as though they belonged to different "sexes," and the same is true for the minus ("male") strains of these varieties.

Mating Types in Paramecium. Paramecium and other ciliate protozoans are remarkable because of a peculiar duality of the nuclear apparatus. They carry a so-called *micronucleus* which is diploid and undergoes regular mitotic and meiotic divisions and a *macronucleus* which, though it arises as a division product of a micronucleus, is apparently polyploid and divides

amitotically. A paramecium may divide for many generations asexually, forming a clone. The sexual process occurs at *conjugation*, during which individuals form pairs; in both members of these pairs the macronuclei disintegrate and the micronuclei undergo meiosis. One of the nuclei resulting from meiosis stays in the individual in which it is formed, while the other nucleus migrates to the other individual of the pair and there fuses with the stationary nucleus. In such a way, conjugating individuals undergo reciprocal cross-fertilization, and each acquires a diploid nucleus resulting from sexual fusion; one of the division products of this fusion

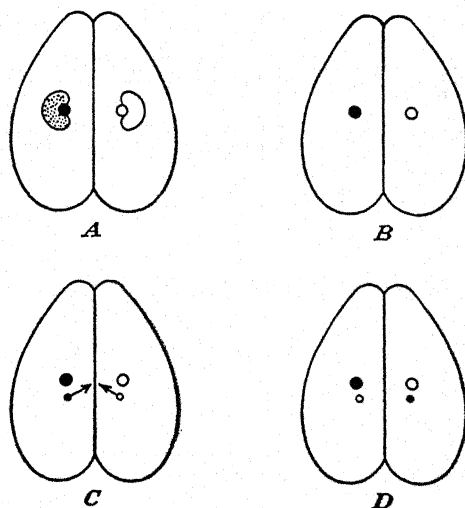


FIG. 167. A simplified diagram of cross-fertilization in *Paramecium*. *A*, two individuals, each with a macronucleus and a diploid micronucleus; *B*, macronuclei have disintegrated, micronuclei have undergone meiosis, and are now haploid; *C*, micronuclei divide into egg and sperm nuclei; *D*, egg and sperm nuclei from different animals about to fuse and form new diploid micronuclei. (After Sonneborn.)

nucleus gives rise to the new micronucleus and another to the new macronucleus (Fig. 167).

Although individuals that undergo conjugation are morphologically so similar that neither of them can be labeled a female or a male, they nevertheless differ genetically. Sonneborn, Jennings, Kimball, and their collaborators have discovered that several species of *Paramecium* and one species of *Euplotes* are divisible into mating types between which, but not within which, conjugation takes place.¹ A species may consist either of

¹ *Paramecium aurelia*, *P. bursaria*, and several other named "species" of infusoria are subdivided into noninterbreeding "varieties," which are in turn subdivided into interbreeding mating types. The so-called varieties are evidently reproductively isolated species which happen to be morphologically too similar for ready recognition under a microscope.

two or of several, up to eight, mating types. If the mating types are compared to sexes, the species which contain more than two mating types exemplify the phenomenon of *multiple sexuality*, which is known also in some algae and fungi. In species which have more than two sexes, repre-

TABLE XLIII. THE SYSTEM OF BREEDING RELATIONS IN *Paramecium bursaria*
After Sonneborn

+ indicates the occurrence of conjugation, - indicates that no conjugation occurs, in mixtures of the two mating types represented on the corresponding row and file

Variety	Mating type	I				II								III				IV		V	VI			
		A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
I	A	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	E					-	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-
	F					+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	G					+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	H					+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	J					+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	K					+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-
	L					+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-
	M					+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-
III	N													-	+	+	+	-	-	-	-	-	-	-
	O													+	-	+	+	-	-	-	-	-	-	-
	P													+	+	-	+	-	-	-	-	-	-	-
	Q													+	+	+	-	-	-	-	-	-	-	-
IV	R																	-	+	-	-	-	-	-
	S																	+	-	-	-	-	-	-
V	T																			-	-	-	-	-
VI	U																				-	+	+	+
	V																				+	-	+	+
	W																				+	+	-	+
	X																				+	+	+	-

sentatives of any two of them can mate, but representatives of any one usually cannot. The mode of inheritance of the mating types in *Paramecium* is rather complex and varies in detail from species to species. The variations are explained in a satisfactory manner by the differences in the behavior of the nuclei during conjugation and during the peculiar process

of reconstruction of the nucleus known as endomixis which occurs in some ciliates. The important point is, however, that the mating type of an individual is determined by its genes, which are inherited in a regular manner through the chromosomes. Certain mating types can be either homozygous or heterozygous; homozygotes produce by asexual reproduction or by endomixis only individuals of the same mating type, while heterozygotes may under certain conditions give rise to strains of different mating types. Other mating types are always homozygous and can give rise to individuals of different mating types only after conjugation, which, of course, involves union of individuals of different mating types. Whether the mating types are determined by alleles of a single gene or by many linked genes is unknown, but in any case these genes are inherited as a unit (Table XLIII).

Sex in Fungi and Bryophytes. Blakeslee showed that in the bread mold *Mucor* and its relatives zygosporangia (resulting from sexual fusion between hyphae) are sometimes produced by the union of hyphae from the same mycelium, or individual. Such forms he termed *homothallic*. In most cases, however, zygosporangia are formed only when two distinct individuals (mycelia) come together; and in such cases the mycelia must be physiologically different as to sex, or *heterothallic*. Within a given heterothallic species it is possible to assign every individual to one of two types, arbitrarily designated as "plus" and "minus." Two plus individuals will not unite sexually, nor will two minus individuals, but when a plus and a minus mycelium are in contact, zygosporangia are produced by fusions between them. It is thus easily possible to determine the type of any unknown individual by growing it in contact with a known plus race and a known minus one.

Many of the basidiomycetes have plus and minus uninucleate mycelia. Anastomoses between these give rise to a binucleate mycelium, which ultimately produces the sporophore. The two strains are probably separated again in the cell divisions immediately preceding the formation of the basidiospores, from which new uninucleate plus and minus mycelia again arise. More than two distinct types have been reported in a number of basidiomycetes.

The actual point in the life cycle at which sex determination takes place has been located much more definitely in some of the ascomycetes through the work of Dodge. In several species of *Neurospora* (the pink bread mold) the mycelia are uninucleate and thus presumably haploid and have been clearly shown to be heterothallic. Through fusion between two mycelia of opposite type, a diploid perithecius develops, and in this the asci, or spore cases, are produced. Each ascus is originally uninucleate, but in most species, by three successive divisions of this nucleus, eight spores are ultimately produced. Since these spores retain their original order in the

ascus, it is possible from their positions to determine the lineage of each and thus to distinguish the groups separated at the first, the second, and the third cell divisions in the ascus. By a delicate technique Dodge has been able to isolate each of the eight spores of an ascus and to grow from

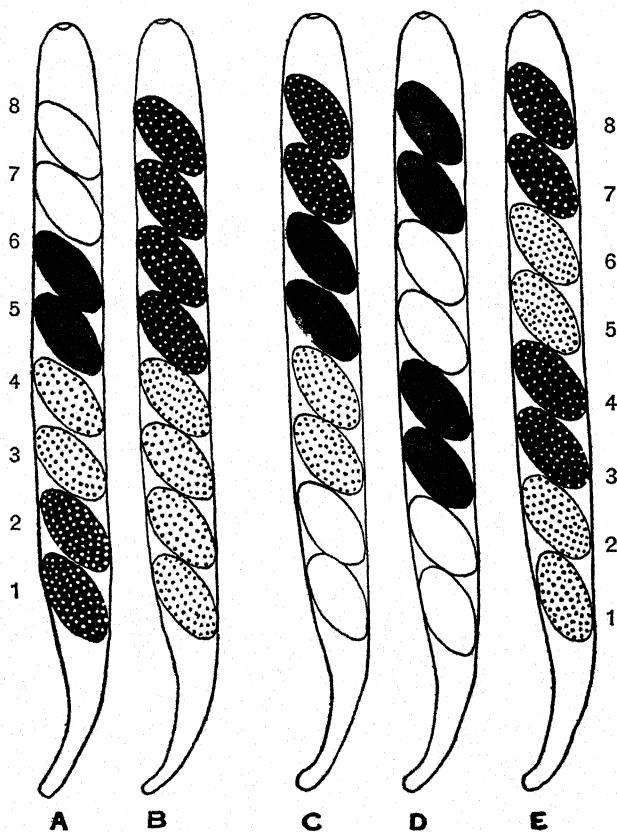


FIG. 168. Segregation in asci of *Neurospora sitophila*. Black and white spores indicate segregation for sex; dotted and plain indicate presence or absence of conidia. Thus in C, segregation for sex occurred at the first division and for conidia formation at the second division. (From Dodge.)

each a mycelium. When this is done, it is found that sometimes the first four spores (1, 2, 3, and 4 numbered from the bottom, Fig. 168) give one sexual reaction (plus, let us say) and the other four (5, 6, 7, and 8) an opposite one, indicating that the segregation of the two sexual types were accomplished at the *first division* (Fig. 168, B, C). In other cases, however, spores 1, 2, 5, and 6 are plus, and 3, 4, 7, and 8 are minus, indicating that the products of the first division still possess *both* sexual potentialities

and that these are not segregated until the second division (Fig. 168, A, D, E). According to Lindegren, whether the sex gene segregates at the first or at the second meiotic division depends upon the location of that gene in the chromosome with respect to the centromere. As shown in Fig. 94 (p. 219), the centromeres of homologous chromosomes always segregate at the first meiotic division. But the maternal and paternal alleles of a gene which lies in a chromosome at a distance from the centromere may segregate either at the first or at the second meiotic division, depending upon whether a chiasma has or has not been formed between the locus of the gene and the centromere.

Bryophytes. In some of the bryophytes both male and female sex organs are produced on the same plant (the gametophyte), and all the spores carry the potentialities of both sexes. Many other species, however, are clearly heterothallic, the plants all being either strictly male or strictly female. The Marchals have shown that in these cases about half the spores in a sporogonium (which arises from a fertilized egg) develop into male plants and about half into female ones, thus indicating that the segregation for sex takes place at spore formation. Regeneration experiments carried on by the Marchals and by Fritz von Wettstein have also shown that protonemata arising from any cell of a male plant will produce nothing but male plants and from a female nothing but female ones but that those regenerating from the tissue of the sporogonium (which is diploid) give rise to *bisexual plants*. Determiners for sex are thus sharply separated in the unisexual gametophytes, come together in the diploid sporophytes, and are again separated at sporogenesis, presumably by the meiotic divisions.

The first clear case of a chromosomal mechanism associated with sex determination in plants was also found in this group. In the heterothallic liverwort *Sphaerocarpos*, both Douin and Allen showed that the four spores resulting from the division of a single spore mother cell give rise to two female and two male gametophytes. Allen later found that in every cell of the female gametophyte there are seven chromosomes, evidently homologous with seven in the cells of the male gametophyte, and in addition one large X chromosome, the homologue of which, in the male, seemed to be a very much smaller Y chromosome (Fig. 169). The diploid sporophyte is thus $14 A + X + Y$, the female gametophyte $7 A + X$, and the male $7 A + Y$. Here the segregation of the XY pair at meiosis parallels that of the sexes, suggesting that femaleness is associated with the X and maleness with the Y. These chromosomes evidently carry other determiners than those for sex, however, since Allen has found that the character of separate spores (as opposed to their persisting union in tetrads) is inherited only through the female parent and is thus presumably determined by a

gene in the X chromosome, a condition comparable to that of a sex-linked trait in animals. Other examples of heterochromosomes associated with sex differences, though in a somewhat more complicated fashion, have been found among other liverworts.

Sex in Higher Plants. Most seed plants are hermaphrodites, having heterothallic gametophytes arising from two types of spores produced on the same sporophyte. The spore-bearing structures are now termed flowers, the microsporangia (bearing microspores or pollen grains) being borne on stamens and the megasporangia (bearing megaspores) on carpels. Among most species both these structures occur in the same flower, which is thus bisexual or hermaphroditic. In many cases, however, stamens and carpels are borne on different flowers but on the same plant, which is then

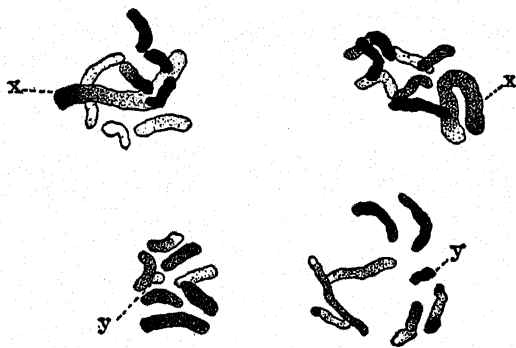


FIG. 169. Chromosomes of *Sphaerocarpos*; from female gametophyte, above, and from male, below. Sex chromosomes indicated by X and Y. (From Allen.)

termed *monoecious*. Numerous intermediate conditions between hermaphroditism and monoecism are known. It is significant that the sporophytic plant is capable of producing both microspores and megaspores, however these may be distributed among individual flowers, and that the primary sexual difference thus seems to be without a genetic basis but to arise in genetically identical material during the development of the sporogenous tissues.

Among a few of the seed plants the two types of spores are borne not only in different flowers but on different plants, the primary sexual difference, actually manifest in the gametophyte, thus being pushed back into the sporophyte, where it has a definite genetic basis. In such *dioecious* species it is possible to speak of "male" and "female" plants in the sense that they bear spores which will develop into male or female gametophytes, respectively.

The genetic difference between these two types was first studied by

Correns in the early years of this century. In the genus *Bryonia* he found that, if a female of *Bryonia dioica*, which is dioecious, was pollinated by the monoecious species *B. alba*, all the offspring were female plants. If *B. alba*, on the contrary, was pollinated by *B. dioica*, about half the offspring were male and half were female. From this Correns inferred that in the dioecious species the eggs are of one sort as far as sex determination is concerned but that the male gametes are of two kinds, half male in tendency and half female. In other words, the male plant seems to be heterozygous for sex (XY) and the female homozygous (XX).

Studies by Baur, Shull, Winge, and others on dioecious species of the genus *Melandrium* (*Lychnis*), a member of the pink family, still further strengthened this hypothesis by demonstrating several cases of sex-linked



FIG. 170. Adult rosettes of *Lychnis dioica*; on the left a plant of the normal form, *typica*; on the right a plant of the narrow-leaved form, *angustifolia*. (From Shull.)

inheritance of the male-heterogametic type. Thus in the dioecious *Melandrium album* (*Lychnis dioica*) there is a broad-leaved form and a narrow-leaved one (Fig. 170). This difference is apparently due to a pair of genes, *Bb*, in the X chromosome. The narrow type is recessive and is lethal in pollen grains, the *Xb* grains dying. Thus a female plant homozygous for broad leaves crossed with a narrow-leaved male will produce all broad-leaved male offspring:

$$\begin{array}{l} (XB)(XB) \times (Xb)Y \\ (XB)Y \text{ broad-leaved male} \\ (XB)(Xb) \text{ not formed} \end{array}$$

If the female parent is heterozygous, the offspring will likewise all be male,

but half will be broad-leaved and half narrow-leaved. Thus:

$$(XB)(Xb) \times (Xb)Y$$

$(XB)(Xb)$ not formed
 $(XB)Y$ broad-leaved male
 $(Xb)(Xb)$ not formed
 $(Xb)Y$ narrow-leaved male.

A heterozygous female crossed with a broad-leaved male, on the contrary, produces both male and female offspring, the latter all broad-leaved, and the former half broad and half narrow, thus:

$$(XB)(Xb) \times (XB)Y$$

$(XB)(XB)$ broad-leaved female
 $(XB)Y$ broad-leaved male
 $(Xb)(XB)$ broad-leaved female
 $(Xb)Y$ narrow-leaved male

Winge has found other traits in this species which are inherited in somewhat the same fashion. These evidently conform in their essential features

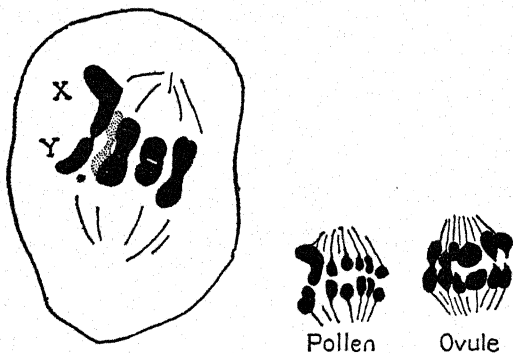


FIG. 171. Sex chromosomes in Melandrium. (After Běllár.)

to the types of sex-linked inheritance found in *Drosophila* and many other animals.

The cytological basis for sex differences in dioecious plants has also been determined in many cases. Female plants of *Melandrium*, for example, have been shown to possess two large X chromosomes, and male plants an X and a much smaller Y (Fig. 171). A similar distribution of heterochromosomes has been found in over 50 other species of dioecious plants, as in *Elodea*, *Rumex*, and *Humulus*. In many other dioecious forms, however, such as *Bryonia*, there are no visible chromosome differences between the sexes.

Westergaard in Denmark and Warmke and Blakeslee in the United

States independently studied sex inheritance in polyploid *Melandrium album*. The diploid female plants have 24 chromosomes, of which 22 are autosomes and 2 are X chromosomes ($22 + 2X$); diploid males have the same chromosome number but they have one X and one Y chromosome ($22 + XY$). Tetraploid plants are of three types: $44 + 4X$ females, $44 +$

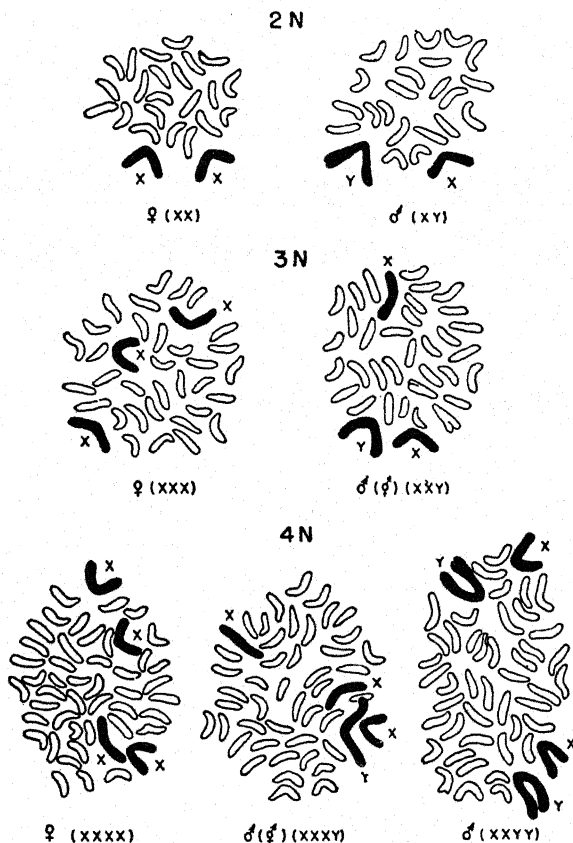


FIG. 172. Chromosome complements of diploid (top), triploid (center), and tetraploid (bottom) plants of *Melandrium*. (From Warmke.)

$2X\ 2Y$ males, and $44 + 3X\ 1Y$ males (Fig. 172). This shows that, in contrast to *Drosophila*, the Y chromosome of *Melandrium* is definitely sex determining and carries genes for maleness. One Y chromosome is sufficient, even in the presence of three X's, to make a tetraploid plant male. Triploid plants, obtained by crossing the tetraploids and diploids, are $33 + 3X$ females and $33 + 2X\ 1Y$ males. According to the *Drosophila* model of sex determination, these latter plants, having two X's and three

sets of autosomes, should be intersexes, but in *Melandrium* the presence of the Y chromosome makes them males. Intersexual (or more precisely hermaphroditic) *Melandrium* plants have been obtained by fragmentation of the Y chromosome. It follows, then, that the Y chromosome carries at least two genes for masculinity.

Transition between Hermaphroditism and Bisexuality. It happens in many groups of organisms that rather closely related forms differ in that some of them are monoecious and others dioecious. Thus, dioecious genera of higher plants occur in various families which include also mono-



FIG. 173. Normal hermaphroditic maize, left; silkless (male), center; tassel seed (female), right.

ecious genera. This fact suggests that changes from hermaphroditism to bisexuality, and perhaps also from bisexuality back to hermaphroditism, have occurred independently in the evolution of different groups of organisms. Jones has reproduced such a transition experimentally in maize.

The maize plant is ordinarily monoecious, the terminal inflorescence (the tassel) being male, the lateral inflorescences with pistils, styles (silks), and stigmata being female. Mutant genes are known which suppress the development of the silks, and plants homozygous for such a gene function only as males; other mutant genes cause the production of female flowers and seeds in the tassel, and plants homozygous for such a gene function chiefly as females, although the tassel may also produce functional pollen. Jones crossed a silkless plant, *sk sk*, as male parent with tassel-seed plant,

ts ts, as female. The F_1 plants, *Sk sk Ts ts*, were normal hermaphrodites with the usual tassels and cobs, that is, each mutant type contained the normal allele of the other, and reversion to the normal monoecious condition occurred. When these F_1 's were inbred, the F_2 consisted of normal hermaphrodites, silkless (male), and tassel-seed (female) plants in a ratio of 9 hermaphrodites:3 males:4 females. Breeding tests showed that the tassel-seed females were of three types: *Sk Sk ts ts*, *Sk sk ts ts*, and *sk sk ts ts*; that is, *sk* had no effect in the presence of *ts ts*. The results of crossing such double recessive females with silkless males heterozygous for *ts* produced exclusively males and females as shown below:

$$\begin{array}{c} sk\ sk\ ts\ ts\ (\text{♀}) \times sk\ sk\ Ts\ ts\ (\text{♂}) \\ | \\ sk\ sk\ ts\ ts\ (\text{♀});\ sk\ sk\ Ts\ ts\ (\text{♂}) \end{array}$$

Continued breeding of these types gave always this clear segregation, that is, this stock had become dioecious. The differences between functionally male and functionally female plants in this family are determined by the segregation of a single gene pair *Ts ts*. Since *ts* is located in chromosome I, this chromosome has become in effect a sex chromosome, and since the male is heterozygous for the most effective locus in this chromosome, it resembles closely the male heterogamety found so frequently in animals and other dioecious plants.

How easily changes in the sexual mechanism may occur in forms in which bisexuality is phylogenetically a rather recent condition is also shown by the work of Gordon and Kosswig on the fishes *Platyopocilus* and *Xiphophorus*. In the latter species a genetic mechanism of sex determination is absent or so weak that an individual may become a female and a male consecutively during its lifetime. In *Platyopocilus*, however, sex is determined by chromosomes, and this chromosomal mechanism is decisive also in the hybrids of the two forms. Furthermore, Gordon discovered that the "domesticated" strains of *Platyopocilus maculatus* (which have been kept by aquarists for many generations) are XX in females and XY in males, that is, only one kind of eggs but two kinds of spermatozoa are formed. The strains of the same species derived from fish recently collected in their natural habitats in Mexico are, on the other hand, XY in females and XX in males. The mechanism of sex determination must consequently have been altered since the species was domesticated.

Evolution of Sex Chromosomes. It is very probable that in organisms in which the bisexual condition has phylogenetically recently evolved from a hermaphroditic one the sex-determining chromosomes (X and Y) carry much the same gene loci and differ only in a single or in few genes that act as sex differentiators. The changes described above in *Platyopocilus*

certainly suggest that sex in these forms has a rather simple genetic basis. But as bisexuality becomes established in an evolutionary line, the differences between X and Y chromosomes become progressively greater.

As pointed out particularly by Muller and Darlington, crossing over between X and Y becomes restricted to only part of the length of these chromosomes (*the pairing segment*), while other parts which carry the sex-differentiating genes undergo no crossing over (*the differential segment*). The genes borne in the differential segment of the Y chromosome, provided that they play no important part in sex determination, gradually degenerate by mutation or become lost. This is because the Y chromosome is transmitted only from father to son (or from mother to daughter) and never becomes homozygous in any individual. Recessive lethal mutations or gene deficiencies that are eliminated in chromosomes other than the Y in homozygous individuals are sheltered from natural selection when they occur in the Y chromosome. Such losses of genes lead to the situation exemplified by the Y chromosome of *Drosophila*, which is genetically nearly inactive, or "empty." Or the Y chromosome may be lost altogether, as in some grasshoppers and other insects. At the same time, the single sex differentiator in the X chromosome is gradually replaced by numerous cooperating sex genes, as has been shown in *Drosophila* (see p. 384). The human X and Y chromosomes seem to be at a stage somewhere between those exemplified by *Platypocilus* and *Drosophila*. The pairing segments are still fairly large and contain several genes, which, accordingly, have alleles both in the X and in the Y chromosomes. The differential segment of the X is also large and contains all the genes which show a typical sex-linked inheritance; that of the Y is small and apparently contains only a few active genes.

The Effects of Hormones. The general theory of sex as the result of a balance between opposed tendencies has been successfully applied to the conditions found in many animals. In the higher animals and especially in vertebrates the secondary products of the sex glands—the sex hormones—may very considerably alter the final characters. In the fowl, for example, early removal of the ovary from a "genetic" female may reverse some of the normal processes and result in the development of male comb, plumage, and behavior and even in the appearance of a testis. An extreme case of sex reversal of this sort has been reported by Crew. A hen, said to have laid fertile eggs, ceased laying, developed male comb and plumage, crowed, finally functioned as a male, and became the father of two chickens. Its ovary had apparently been destroyed by disease and replaced by two testes. A similar case has been reported in pigeons, and such reversals of sex are known to occur normally in certain amphibia, fish, and lower forms in which an individual may begin life as a functional female and later become a functional male.

The effect of early hormonal influence on sexual characters in cattle has been studied by Lillie and others, who have shown that, where twins of opposite sex are born, one is a normal male while the other is usually a sterile female with many malelike traits—the so-called “freemartin.” The evidence from early development shows that, through anastomosis of blood vessels, the blood of one embryo, with its hormones, may enter the blood stream of the other. In this case the male hormones seem to influence the development of the female embryo in a male direction.

In plants, also, the development of the sex organs may be altered by influences from the environment which operate through channels like those of the animal hormones. In the dioecious hemp plant, for example, changing the length of day to which the plant is normally exposed may result in production of male flowers on female plants, and vice versa.

Such cases remind us that sexual differences, like other phenotypic differences, depend upon the reaction of a genotype to the conditions which it encounters during development. Where the sex-chromosome mechanism is well established, as in most animals, sex is *determined* at fertilization, in the sense that the preponderance of genes leading development toward one sex type is decisive, although, as we have seen from instances of sex reversal and of intersexuality, the preponderance may be overcome by genes of the opposed type or by environmental factors. The means by which the sex genes influence the development of sexual characters, that is, the problem of sex differentiation, is thus a part of the more general problem of how genes influence development. In the demonstration of the interaction of many genes, some tending to influence a character or reaction in one direction, while others tend in an opposite direction, the balance theory of sex-determination reveals a more general mechanism, known as genic balance, by which the genes influence the adult characters. Some of the evidence and ideas concerned with the developmental origins of phenotypes are reviewed in the next chapter.

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CHAPTER XVI

GENETICS AND DEVELOPMENT

The principles of genetics, as set forth in the opening chapters of this book, have been derived chiefly from a study of genes and of chromosomes at the time of meiosis and fertilization. However, since genes are known by their effects, the laws of their transmission are in fact inferences from the distribution of phenotypes, that is, from the appearance of the differentiating characters. In the higher plants and animals, most of these characters do not arise directly from the genes but result from a chain of developmental processes, initiated by a gene or genes, and leading through interactions, with events controlled by other genes and factors of the internal and external environment, to the final phenotype. A question which recurs continually in genetics is how the genotype is related, causally, to the developed character. Through what means do the thousands of individual genes of the fertilized egg cell produce the phenotype of the multicellular individual with its complex organization of specific structures and functions, its behavior, and individual peculiarities?

Not only is this question of great importance as a necessary part of our knowledge of the relation of the genes to development, but since the effects which genes have on individuals determine their chance of survival, their distribution, and hence the character of the species, it is necessary to a full understanding of the relation of genes to evolutionary processes.

In considering this question we may distinguish, for convenience in discussion, two aspects of it which are closely interrelated. One concerns the mechanisms by which the hereditary characters reach their final form. In seeking for principles governing this relationship of genes and characters, we are led to trace the differences between specific phenotypes such as those of AA , Aa , and aa back to their ontogenetic origins. This involves an analysis of the character during development, and this type of study has been referred to, since Haecker first used the term in 1918, as phaenogenetics or developmental genetics. In practice phaenogenetics is limited to the comparison of structures or processes or substances in individuals with known genetic differences, since in general only gene differences, not genes themselves, can be studied by this method.

The other aspect of the main question concerns the mode of action of the gene, that is, the mechanisms by which genes initiate the reactions

which give rise to specific substances, to developmental processes, and thence to phenotypes. Studies directed toward the elucidation of this question are often referred to as being concerned with *gene physiology* or *gene action*, which is the subject of the last chapter.

Of course no sharp distinctions can be drawn between physiological and developmental genetics. Both are concerned with one main process, in one case approaching it from its beginning in the gene, in the other working backward from the characters or reactions or substances found in development toward the gene. It is nevertheless true that some methods and materials are better adapted to the study of one rather than the other aspect of the gene in relation to its effects.

In this chapter we shall describe some of the problems and results of studies which are concerned specifically with the relation between genes and development. In general, the data to be considered have been derived from direct observations of gene-controlled processes during development although in some cases they rest on inferences from the appearance of completed characters.

Gene and Character. The genes and the characters are parts of one functioning whole, the organism, in which the parts are interdependent, so that genes which affect one part are likely also to influence others and thus to have manifold effects. Often the characters in many parts of the animal or plant are due to the effect of the gene on a single widespread process, such as the failure of pigment formation in albinism or the differences between the mutant *aa* and the wild type of the flour moth, *Ephestia Kühniella* (Table XLIV, p. 419). But in many other cases the multiple effects have no obvious relation to each other, and they are then referred to as *pleiotropic*. Thus the mutation yellow in the house mouse acts as a lethal when homozygous, and when heterozygous it affects the color, the extent of white spotting, the fatness, and the fertility of the animal. In most cases the possibility cannot be excluded, because of the difficulty of studying the earliest stages of development, that pleiotropic effects of mutant genes also trace to some single altered process. The significant fact, however, is that genes produce their effects not as isolated elements, each related to one character, but as part of an integrated system which is likely to undergo widespread changes when one of its constituents is altered.

Expressions often employed by geneticists such as "the gene for white eyes" or "a gene for hair form" should consequently not be taken too literally. They are used as abbreviations for some such lengthy expression as "a gene, certain mutational alterations of which so influence the development of the organism, that individuals with the original and the altered gene alleles differ in many ways, among which the whiteness of the eyes is most easily observed." The genes are not the representatives in the gam-

etes of definite body parts, such as eyes or hair, as some earlier biologists thought. Instead, the genes determine, *as a group*, the development of the organism from fertilization to death. We learn about the existence of a gene when it changes by mutation, and we observe the segregation of certain phenotypic differences in the offspring of hybrids between the original and mutant forms. ✓

It is frequently observed that a gene does not always produce the same character effect in all of the individuals in which it is present. Thus, of all members of a *Drosophila* population homozygous for the mutant gene "abnormal abdomen" only about 15 per cent show the kind of difference from the normal type for which the mutant is named. This mutant is said to have a *penetrance* in the population of 15 per cent. Similarly, the dominant mutant *Lobe*⁵, when heterozygous, shows a penetrance of about 75 per cent under certain conditions, and this can be changed by changing the culture conditions. Often the penetrance of one gene is increased or decreased by other genes, which thus act as modifiers or suppressors. Whether or not a gene produces a detectable change in phenotype thus depends on the environment and the genotype as a whole, and penetrance merely describes the frequency with which overt expression is reached and provides no explanation of the fact, which has to be subjected to analytical study. Like dominance, penetrance appears to be due to the interaction between the gene and the system of which it is a part.

Phenocopies. Since the characters of organisms arise as responses of the genotype to the environmental conditions, it is not surprising to find that characters change when an external condition is changed in much the same way in which they change when a gene mutates. In cases in which the mutant change has been observed first and then an imitation, or mimic, of it is found to follow an environmental change, the mimic is sometimes called a *phenocopy*. In fowls, for example, there is a mutant gene which under certain conditions interferes with the development of the rump, including caudal vertebrae, tail feathers, and oil gland (Fig. 174). The same character, rumplessness, may be produced as a phenocopy by treating normal eggs (those without any rumpless gene) with insulin before incubation. Other environmental agents such as shaking also produce rumpless phenocopies, which behave as nonhereditary modifications and breed as normal fowls do. Such phenocopies arise occasionally as sporadic variants without treatment in flocks of normal fowls and are probably due to accidental effects on the early embryo (Fig. 175).

Goldschmidt, who introduced the term phenocopy in 1935, pointed out the importance of phenocopies in analyzing development, since he supposed that the duplication of mutant effects by environmental agents applied at specific times might be used to investigate when and how the mutant gene

changes the velocity of certain processes in development. Thus Goldschmidt and others have produced phenocopies of many of the mutant types of *Drosophila* by subjecting the larvae for short periods to higher than normal temperature and have attributed the results to sudden changes in the rates of developmental processes. Goldschmidt had already suggested that mutant genes act in this same way.

Landauer and his associates have succeeded in producing phenocopies of several mutations with skeletal effects in the fowl by injecting eggs of normal genotype with insulin before incubation or during early development.

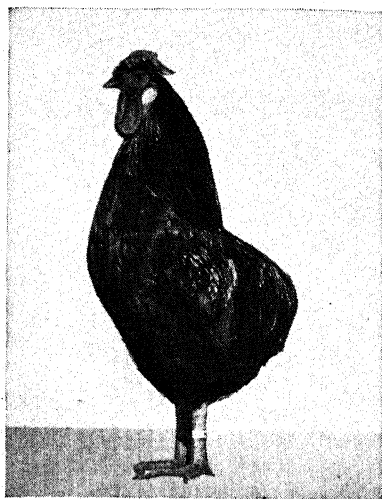


FIG. 174.



FIG. 175.

FIG. 174. A genetically rumpless fowl, heterozygous for a mutant gene (*Rp rp*).

FIG. 175. A fowl with a sporadic phenocopy of rumplessness. This bird did not transmit the character.

The effects vary with the time of injection: in the earliest stages (up to 72 hours) rumpless phenocopies are obtained; later, modification of genetically polydactylous embryos toward normal; and still later (fifth and sixth day), injection of insulin produces micromelia (shortening of limb) and abnormalities of upper beak and skull like those associated with several mutant genes. Insulin effects, which arise after the establishment of circulation, are accompanied by hypoglycemia, a symptom of disturbed carbohydrate metabolism. It is interesting that nicotine amide, which may act as part of a coenzyme in cell respiration, serves to counteract the effect of insulin and thus to prevent these insulin-induced phenocopies. It remains to be seen whether nicotine amide will also counteract the effect of a rumpless gene. The developmental pathways leading to a given charac-

ter may be elucidated by future work of this kind and the effects of mutant genes and their normal modifiers equated with substances and reactions with known physiological effects.

Genic Balance. Another general fact about the relations of genes and characters has been embodied in the concept of genic balance. Bridges, in attempting to explain the "exaggeration" effects of recessive mutant genes when opposite to a deleted or deficient piece of chromosome, assumed that of the many genes influencing a character some tend to accentuate and others to diminish the character; and its actual condition thus represents the point of balance between the opposed tendencies. This view gained considerable support when applied in the balance theory of sex determination (p. 382) and is a necessary assumption in explaining the developmental effects of chromosomal aberrations such as heteroploids which differ from the normal type, not by mutated genes, but by the ratios or relative dosages of unchanged genes.

Typical examples are found in cases in which the effects of different numbers of such unmutated genes can be compared, as in polyploids or heteroploids in which the ratios between different sets of chromosomes have been modified by chromosomal mutations. In the Jimson weed, *Datura*, the wild type has 12 pairs of chromosomes. When the members of any one pair are in excess, certain characters in all plant parts regularly differ from this normal type. The differences produced are characteristic of the particular chromosome which is in excess and which thus modifies, in one direction or another, the normal genic balance exhibited by the diploid. Thus, the mutant "Globe" is trisomic ($2n + 1$) for one chromosome (Fig. 176). In the diploid this produces a change in the shape of the seed capsule and other characters of the plant. If two extra Globe chromosomes ($2n + 2$) are present in the diploid, the mutant effect is greatly enhanced; but the effect of one, two, or even three Globe chromosomes in a tetraploid ($4n + 1$, $4n + 2$, $4n + 3$) is proportionally much less than in the diploid. In these cases, the effect is due to the *ratio* of the extra chromosome to all the others. In the wild type this ratio is 2 Globe: 22 others, or $\frac{1}{11}$; in $2n + 1$ it is $\frac{3}{22}$, or $\frac{1}{7.3}$, whereas in $4n + 1$ it is $\frac{5}{44}$, or $\frac{1}{8.8}$, which is thus nearer to the wild type than the $2n + 1$ heteroploid. The rule is borne out by similar data from other mutants showing that the departure from the wild type is due to the effect of a particular chromosome *relative* to the others. The wild-type condition of the characters represents the stable equilibrium which is disturbed by changes in the ratios of the genes.

This concept makes it possible to understand why a change in a single gene (a point mutation) may and often does produce a greater or more specific change in development than the addition or subtraction of a whole chromosome. The change in ratio between that single locus which has

lost its normal or wild-type allele and the few other loci with similar but opposite effects is much greater than the change involved in adding one each of the whole series of unmutated loci in a normal chromosome set, in which the balance of plus and minus effects is at or near the equilibrium

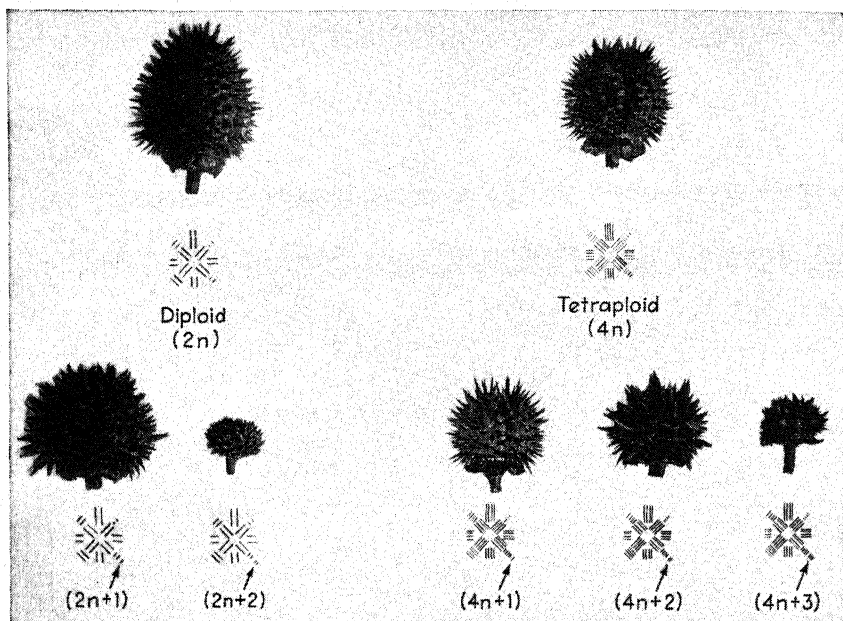


FIG. 176. Chromosome mutants and chromosome balance in *Datura*, as shown by differences in capsule form. Above, at left, the normal diploid type, from plant with twelve pairs of chromosomes. Below (left) the mutant "Globe," in which one set of chromosomes has three members, thus upsetting the normal balance. The effect of this particular chromosome set is evidently to flatten the capsule, for the addition of an extra chromosome to it results in a flatter capsule than the normal. The addition of *two* extra chromosomes (as shown to the right of this) flattens the capsule still further.

Above, at right, a capsule from a tetraploid plant, which has four chromosomes in each set, instead of the normal two. The balance between the twelve sets is thus maintained and there is little difference from the normal in capsule form. The results of the addition of one, two, and three chromosomes to the globe set are shown below. It is evident that the change produced by each additional chromosome is less than it is in a corresponding diploid plant, presumably because the number of chromosomes is greater, the contribution of a single chromosome is less in proportion to the whole, and the balance is therefore less upset. (From *Blakeslee*.)

point. It may also explain the basis of natural selection of particular concatenations of alleles as those which interact most successfully and have the widest margin of safety against disturbance of the developmental equilibrium brought about either by mutation or by changes in the environment.

Direct Relations of Genes and Characters. Although most characters in most organisms probably depend on interaction among the effects of many genes, the degree of interaction will depend on the distance in time and in number and kinds of processes intervening between the first effects of the gene and the appearance of the character. Judgment about the

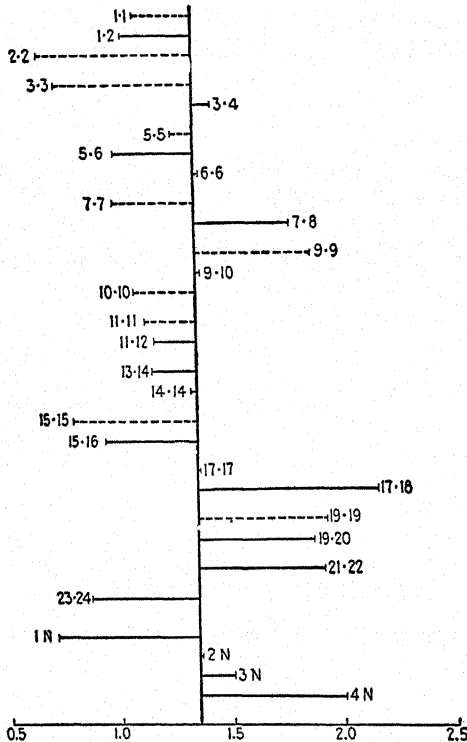


FIG. 177. Genic balance in *Datura* as shown by an anatomical character, the area of the pith (in square millimeters) in the flower stalk. Vertical line indicates mean area of diploid. Areas of primary (solid) and secondary (dotted) mutants (and of polyploid series) are shown by ends of horizontal lines.

degree of interaction and the effects of individual genes will of course depend upon what genic effect, that is, what character change, is measured or observed. In a growing number of cases in which attention has been focused on the biochemical effects of mutant genes or on their relations to antigens or other substances showing marked individual specificity, rather direct relations between gene and substance have been discovered.

1. *Genes and Antigens.* The first case which indicated such a direct

relation between a gene and one of its effects has already been pointed out in connection with the classical blood-group antigens in man. The fact that persons of blood group AB show the full effect of both of the alleles I^A and I^B *without interaction* makes it probable that the chain of reactions leading from the gene to the antigen is relatively shorter than in the case of most other bodily characters. A similar situation exists in the human blood type MN (p. 343), which has the antigens produced by the alleles M and m ; in some combinations of Rh antigens (p. 90); and in some rabbit and cattle antigens. These antigens are specific properties of red blood cells, which in mammals lose their nuclei shortly after the cell is formed; hence it must be assumed that each gene produced its specific antigen directly in the short time before it disappeared from the cell. Although in these cases there is a one-to-one relation between gene and antigen, there are other instances in cattle and birds in which single alleles may contribute to the formation of different antigens. This does not necessarily deny the essential assumption of a direct relation of a gene and a specific substance. A similar interpretation has been applied to the gene-controlled specificities in self-sterility in plants (p. 91).

2. *Biochemical Specificity.* It is often supposed that, where the "character" controlled by the gene is a uniform substance such as a known chemical compound which is synthesized by the organism, there is again little room for gene interaction, although the sources of the materials assembled into the compound and the metabolic system in which it is synthesized probably depend on other genes. There are now many instances in which a gene mutation is known to alter a single step in such a synthesis. The most complete analysis has been made by Beadle and his coworkers on the so-called biochemical mutants of the bread mold *Neurospora*, for which the method of detection has already been described (p. 282). Strains of this fungus have been studied, each of which differs from the normal wild type by a single mutant gene. One of these mutants fails to grow in the absence of the vitamin thiamine, another requires pyridoxine, while another requires para-aminobenzoic acid, or pantothenic acid, or nicotinic acid, or choline. Similarly among other mutant strains, each requires in its food one of the amino acids arginine, lysine, leucine, valine, tryptophane, and several others. It is assumed that in each mutant the gene responsible for some essential step in the synthesis of the substance required has been changed. Since the mutant cannot synthesize an essential food constituent, it will not live unless this substance is supplied to it in its food, and this property of the mutant can be used to determine whether some specific substance, for example, pyridoxine, is present in the medium. If it is, the "pyridoxineless" mutant will grow; if it is not present, this mutant will fail to grow.

In explaining the specificity of the relationship between a mutant gene and a chemical substance it has been assumed by Beadle and his collaborators that each step in the synthesis of the substance is controlled by a gene, which when inactivated by mutation blocks the enzyme reaction responsible for the step affected by the gene. In a number of cases, several of the steps in the synthesis of a substance have been shown to be controlled by separate genes, each one assumed to affect a specific enzyme (Fig. 178).

In a few cases the dependence of a specific enzyme on a gene has been proved. The first proof of this was provided in 1914, when Gross found an enzyme deficiency in the blood of persons with the gene for alkaptonuria. Persons with this rare recessive disease fail to break down, as normal

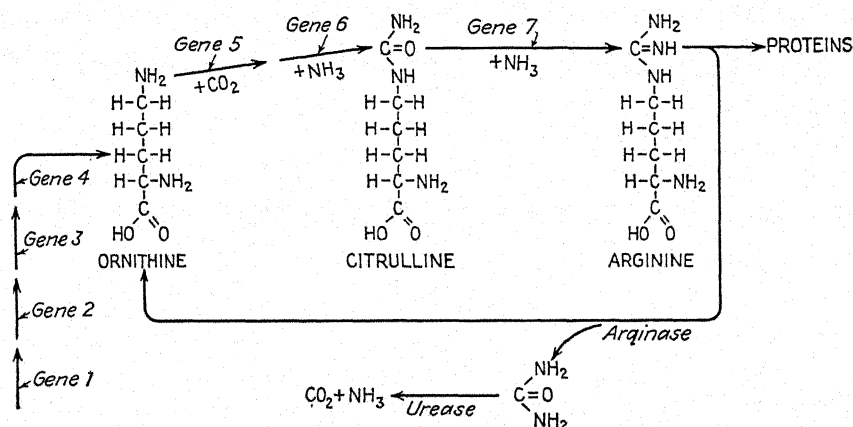


FIG. 178. The assumed effects of seven different mutant genes on the ornithine cycle in *Neurospora*. A mold in which gene 7 has mutated is unable to convert citrulline to arginine and can survive only if arginine is present in its food medium. Mutations in either gene 5 or gene 6 prevent conversion of ornithine to citrulline while if gene 1, 2, 3, or 4 is defective no ornithine is synthesized. (From Beadle, after Srb and Horowitz.)

persons do, the substance known as alkapton, or homogentisic acid (2,5-dihydroxyphenylacetic acid) (Fig. 179), and this is excreted in the urine, which turns black on exposure to air. Normal people have an enzyme which breaks down alkapton, but this enzyme is absent from the blood sera of persons homozygous for the defective gene.

It has recently been established through the work of Følling, Jervis, Penrose, and others that a type of imbecility associated with a defect in metabolism is due to a single recessive gene. Since persons with this mental defect excrete phenylpyruvic acid in the urine, the disease became known as phenylpyruvic oligophrenia, or Følling's disease after its discoverer. Although the disease is diagnosed by the presence of phenylpyruvic acid in the urine, the pathological effects of the gene are due rather to

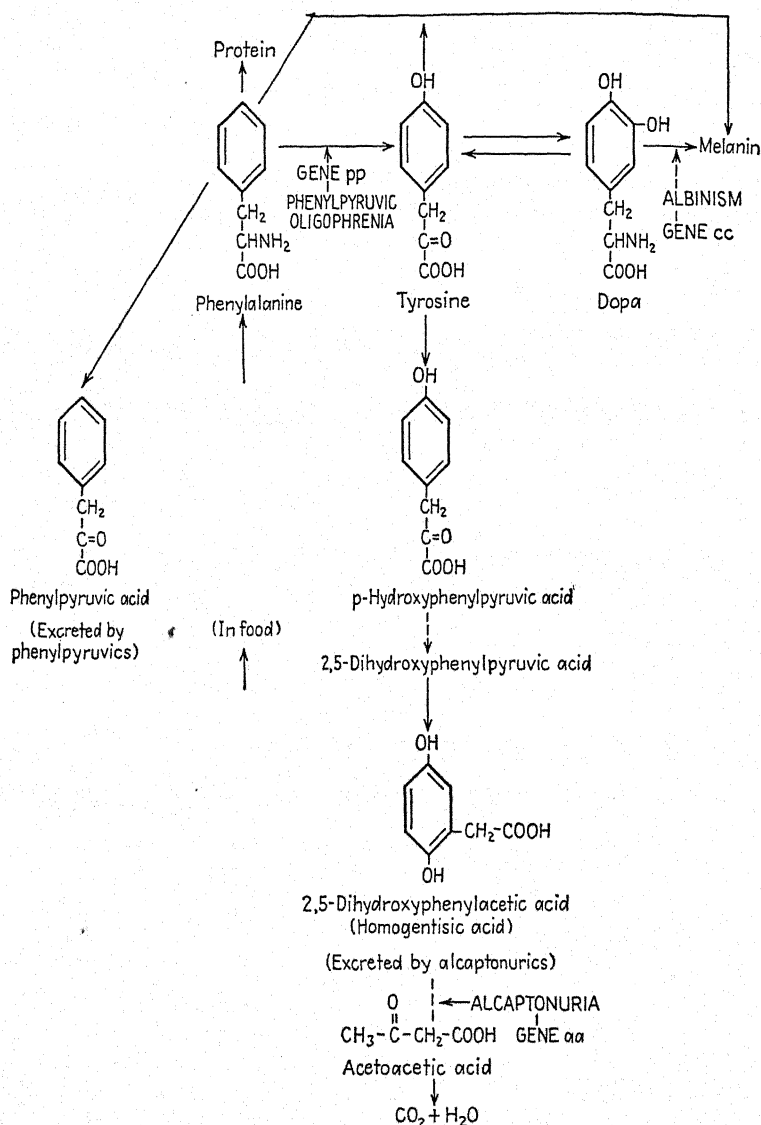


FIG. 179. Scheme of phenylalanine-tyrosine metabolism in man. The conversion of phenylalanine to tyrosine is assumed to be blocked in persons homozygous for the gene for phenylpyruvic oligophrenia. (Modified, after Beadle.)

the inability of those homozygous for it to transform phenylalanine in the normal way. It seems from present evidence that they fail to convert phenylalanine to tyrosine and that this involves inability to hydroxylate the aromatic ring (Jervis, 1947). The probable relationships of the metabolic steps concerned are shown in Figure 179. The reason for the prevalently blond complexions of persons with the disease is also suggested by the break in one of the paths for the conversion of phenylalanine to the dark pigment, melanin. The probable point of failure of the melanin reaction in albinos is also indicated in Figure 179.

A connection between a specific gene and an enzyme has been found in several other cases in animals and plants, and this has led to a general hypothesis of a one-to-one correspondence between genes and enzymes and to a theory of gene action, to be discussed in the last chapter, which considers all genic effects to be by way of enzymes produced or catalyzed by the gene. The relevance of this idea to our present discussion is that, if the "character" being investigated is presence or absence of an enzyme, then in a number of instances it has been shown that the character difference may be produced rather directly by the gene, perhaps in a single step. The existence of this direct connection between a gene and one of its effects of course does not prove that this is the only effect which the gene has, since often other characters are affected which like the mental defect in phenylketonuria have no obvious relation to the one developmental reaction which present methods have enabled us to identify.

INDIRECT RELATIONS OF GENES AND CHARACTERS

Most of the characters heretofore studied by geneticists appear as the end results of a longer or shorter chain of developmental processes occurring at different levels of organization. Some are concerned with substances and processes which occur within or on the surfaces of the cells, such as the antigens and enzymes referred to above, or involve the movements of individual cells; others occur in the tissues as interactions between cells or are responsible for the construction or modification of substances such as hormones or "organizer" influences; still other characters pertain to complex organs such as the flowers of angiosperms or the eyes of vertebrates or to whole systems such as skeleton or muscles, while some are expressed as peculiarities of the whole individual or of groups of individuals as in the inherited patterns of hive or nest building in which a whole population may cooperate (Fig. 180). Such a simple difference in genotype, as between *AA* and *aa*, may cause a divergence between two or more alternative pathways of development at any of these levels, and a divergence at a lower level, as in cellular metabolism, may be expected to cause differences in later links of the chain of developmental processes.

Studies of such indirect relations between character and gene consist of two chief operations, one concerned with *describing* the character difference during development, the other with *analyzing*, usually by experimental methods, the causes of the divergence, which in the ideal case would lead to an identification of the primary effect of the gene difference. In general such phaenogenetic methods may be expected to provide descriptions of developmental changes associated with mutant genes, as the necessary preparation for the more difficult analytical studies. Illustrations of the applications of such methods will be given, together with some of the chief conclusions gained from studies of (1) genetical differences in color in animals and plants; (2) differences in size and form of whole organisms and

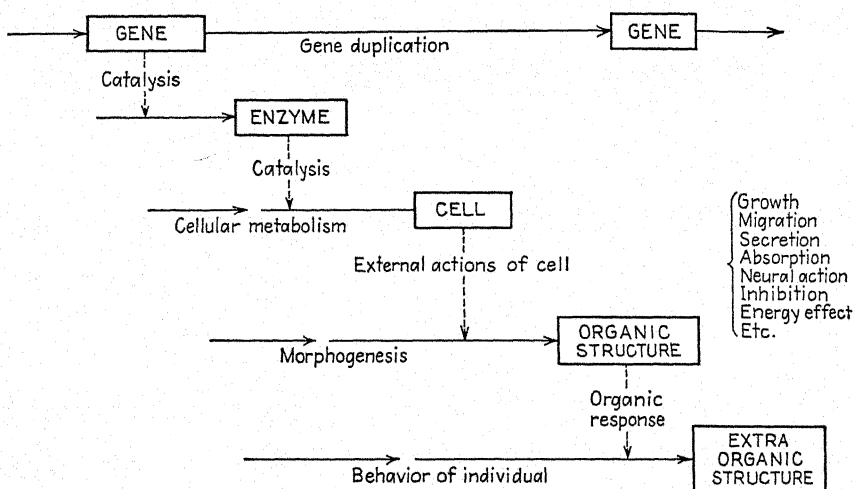


FIG. 180. Diagram illustrating the chain of processes connecting the most immediate effects of a gene with characters at different levels of organization. (After Wright.)

of their parts, which trace to differences in embryological processes; (3) duplication of some of these genetical differences by experimentally controlled environmental differences.

GENIC EFFECTS ON PLANT COLORS

The striking color variation in the flowers of cultivated races of plants such as sweet peas, stocks, primroses, and others long ago attracted the attention of geneticists, who identified a number of the genes responsible. More recently the pigments have been studied by biochemists. Thus we have a better understanding of the chemical basis of genic interaction in the development of flower colors than in any other group of characters. Most of the work has been done with water-soluble sap pigments of two

groups, the anthocyanins and the anthoxanthins. The former are responsible for the various shades or mixtures of red and blue (pinks, purples, magentas, lavenders), while the yellow and ivory colors are due to the related group of anthoxanthins. The anthocyanins and anthoxanthins all contain similar ring structures (Fig. 181) derived from the condensation of sugars. Anthocyanidins differ according to the number and position of hydroxyl groups in the phenyl ring and the methylation of the hydroxyls at 3', 5', and 7. Anthoxanthins differ by having either one hydroxyl at 4' as in the ivory apigenin or at both 3' and 4' as in the yellow luteolin or by the presence of H (in the flavones) or OH (in the flavonols) at position 3. All of the anthocyanins are red in acid solution and blue in alkali. They become bluer in combination with certain colorless anthoxanthins, known as copigments.

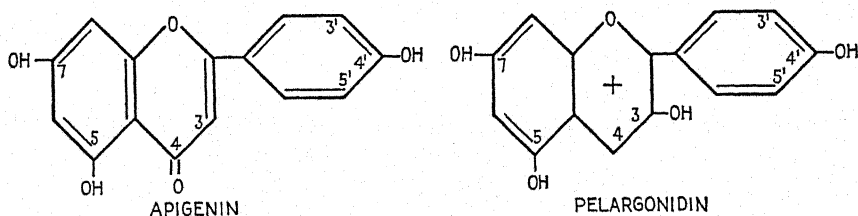


FIG. 181. Structural formulas for an anthoxanthin pigment, apigenin, giving an ivory color (left); and an anthocyanidin, pelargonidin (right). The anthocyanin pigment, pelargonin, is a glycoside of the above, *i.e.*, with a sugar substituted at position 3.

Genes for flower-color differences are known which influence all of these components: presence of pigment and copigment, degree of oxidation or reduction, position of methyl groups, relative acidity or alkalinity, and local distribution of the pigments. In several species—for example, *Dahlia variabilis*—one gene is responsible for the presence of anthocyanin, another for the increased production of the pigment. In others two or more complementary genes must be present before any anthocyanin appears, although anthoxanthin formation is independent of these. In the morning-glory and the snapdragon, the same genes are known to be responsible for both anthocyanin and anthoxanthin pigments since a single recessive gene mutation interferes with the production of any sap pigment, probably by blocking the production or conversion of some precursor substance from which both pigments are derived. When anthocyanin is present, other genes determine modifications in the molecular structure. Genes are known, for example, which control the degree of oxidation of the phenyl ring. Of these, the genes determining hydroxyl groups at 3' and 5' in

addition to 4' and producing delphinidin are dominant to alleles determining oxidation at 3' and 4' (cyanidin) or at 4' only (pelargonidin). In sweet peas genes *A* and *B* together or *A* alone (*AA bb*) produce delphinidin, *aa BB* cyanidin, *aa bb* pelargonidin. The more oxidized pigments turn out to be dominant to the less oxidized ones. One mutant change in flower color from rose pink to salmon pink in *Pelargonium zonale* was shown by Scott-Moncrieff to be due to a gene causing substitution of OH for H at position 3 in the anthocyanidin molecule.

Although the production of these pigments is clearly under the control of specific genes which determine the arrangement of elementary radicals or atoms in a molecule, the relation of gene to pigment character is indirect, probably through the modification of synthetic steps carried out by enzymes in compounds produced as part of the general metabolic processes of the plant and thus influenced by other genes as well.

GENIC EFFECTS ON ANIMAL COLORS

Especially instructive analyses of the way in which color differences in animals are controlled by genes have been made in the case of certain insect pigments concerned in the coloration of the eyes and certain other body parts and in the coat colors and patterns of rodents. Studies of the development of insect pigments led to a general analytical method by which a total developmental process may be broken up into a chainlike series of reactions in which separate steps are controlled by known genes.

Eye Color in the Flour Moth.—In the European flour moth, *Ephestia Kühniella*, Kühn and his coworkers have found mutant genes which affect the colors of various parts of both the larva and the adult. Some of the differences brought about by one such gene substitution are shown in Table XLV.

Caspari transplanted the testes from *AA* or *Aa* larvae to those of the mutant type *aa*. The grafts retained their dark color and caused certain of the characters of the *aa* host to develop the *A* phenotype, that is, *aa* imagoes with *A* testes *implants* had black instead of red eyes; the testes and brain of the host resembled those of the wild type to a greater or lesser degree, and if the transplantation was made into early *aa* larvae, the larval skin and larval eyes in later molts assumed the *A* characters. Similar results were obtained by implanting ovaries or brains from *A* to *aa* larvae. In the reciprocal type of transplantation, *aa* brain and testes implanted into *A* larvae assumed *A* characters. Homotransplantation of *A* tissue to other *A* larvae or of *aa* tissues to other *aa* larvae showed that the changes described above were not due to the operation or injury to tissues.

Kühn and his associates assumed that a substance contained in certain implanted tissues of genotype *AA* or *Aa* could produce their characteristic

effects in host tissues which did not contain this gene. Since the substance was diffusible and separable from *A* tissues by extraction with alcohol and with acetone, they called it *A* hormone. An interesting proof of the dependence of the *A* characters on a circulating substance which *a* animals lack was obtained by implanting *A* tissue into *aa* females, which were then bred to *aa* males. This cross would normally produce larvae with the *a* characters—colorless skin, little pigment in eyes, etc. But eggs from the *a* mothers with implanted *A* tissues produced larvae with the *A* characters. One route by which the gene *A* produced its effects on many tissues was thereby disclosed. The *A* hormone has now been identified chemically as kynurenine, which is also concerned with pigment formation in other insects, as will be described later.

TABLE XLIV

Character affected	Color in wild type <i>AA</i> or <i>Aa</i>	Color in mutant <i>aa</i>
Adult eyes.....	Black	Red
Adult brain.....	Dark brown	Pale red
Adult testes.....	Brown-violet	Colorless
Larval skin.....	Reddish	White
Larval eyes.....	Much pigment	Little pigment

Eye Color in *Drosophila*. A chain of reactions involving at least two different substances concerned in producing the wild-type eye color in *Drosophila* has been demonstrated by Beadle and Ephrussi. In dipterous insects many of the structures of the adult, or imago, such as the compound eyes, legs, and wings, develop from buds which are formed within the late embryo or early larval stages. These are known as imaginal disks. In *Drosophila* the imaginal disk of the compound eye can be transplanted from one larva to another and then can continue its development in the body cavity of the host. When the host has undergone metamorphosis and has emerged as an imago, the implanted eyes can be dissected out from the body cavity and their color characteristics observed. The colors of intact eyes are apparently not altered by the operation itself or by development within the body cavity.

When eye disks are taken from larvae of strains with mutant eye colors (white, peach, pink, carmine, etc.) and implanted into wild-type larvae, or the reverse, the disks usually develop autonomously (Fig.182), that is, they produce eyes with the color of their own genotype and are not affected by the genotype of the host. There are, however, two important exceptions. Eye disks from larvae with the mutant gene *vermillion* implanted into larvae of the wild type and of certain eye-color mutant types develop

not vermilion but wild-type color. Similarly, eye disks from larvae with the mutant eye-color gene cinnabar (an eye color like vermilion) implanted into larvae of the wild type and of some mutant types develop wild-type pigmentation. In both cases something from the host has caused the implant to develop a phenotype corresponding not to its own genotype but to that of its host. Beadle and Ephrussi assumed that the vermilion and

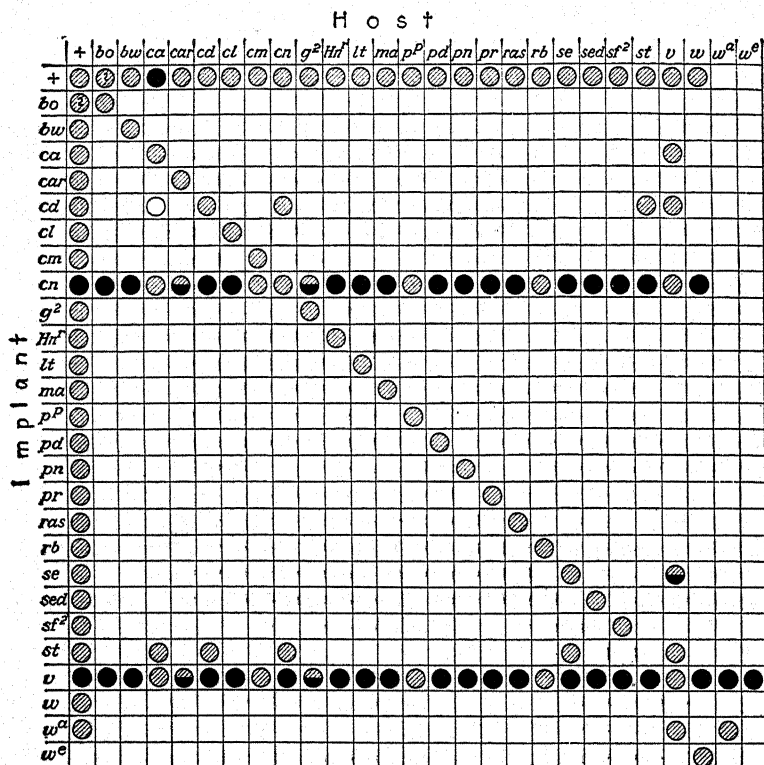


FIG. 182. Diagrammatic representation of the results of eye transplantation in *Drosophila*. Shaded circles indicate autonomous development; for example, brown (*bw*) disks transplanted into wild-type (+) larvae, develop brown color. Black circles indicate nonautonomous development of pigmentation. Circles half black and half shaded indicate that the resulting implant is intermediate in color. (From Beadle and Ephrussi.)

cinnabar mutant flies each lack some substance which is an essential link in the chain of reactions leading to the formation of the wild-type eye pigment, that the eye disks remain sensitive to such substances, and that, in the cases just quoted, the essential substance has been supplied to the disk from the body fluids of the host.

It has been shown that the substance that is lacking in the vermilion

eye is not the same as the substance that is lacking in the cinnabar eye. When eye disks from vermilion larvae are transplanted into cinnabar larvae, the implants develop wild-type pigmentation, showing that the cinnabar host supplies what is lacking in vermilion. However, when eye disks from cinnabar larvae are transplanted into vermilion larvae, the implants develop cinnabar pigmentation; vermilion does not supply what is lacking in cinnabar. It appears then that two substances known as cn^+ and v^+ substances are lacking in vermilion, that one of these, the cn^+ substance, is lacking in cinnabar, and that both v^+ and cn^+ are present in the wild type. Other observations show that the second of the substances (the one that cinnabar lacks) is produced only when the first is present, that is, one substance acts as a precursor of the other. Thus have been demonstrated two related links in a chain of reactions leading to the development of wild-type eye color, and this chain has apparently been broken at an earlier point by the mutation to vermilion, at a later point by the mutation to cinnabar.

These substances are not species specific but are widely distributed in invertebrates. Extracts of *Ephestia* A hormone cause the development of wild-type eye color in the *Drosophila* cinnabar eye; it is the same as the v^+ substance in *Drosophila*. Extracts from *aa* *Ephestia*, however, are without effect on cinnabar; hence it is assumed that the mutations from *A* to *a* in *Ephestia* and from v^+ to *v* in *Drosophila* blocked the eye-pigment reaction chain at the same point, that is, at a stage previous to the formation of the precursor leading to both the v^+ and cn^+ substances.

The v^+ , or A-hormone, substance has been identified as kynurenine, and the effects of genes on the production of the precursors and the pigments are shown in Figure 183. Mutation of v^+ to *v* or *A* to *a* blocks the production of alpha oxytryptophane and thus prevents all formation of brown pigment; mutation to *cn* prevents the conversion of kynurenine into chromogen and similarly yields no brown pigment.

In *Ephestia*, Caspari has shown that *aa* animals have more tryptophane than wild type *AA*; thus it is probable that the oxidative step from tryptophane to kynurenine is blocked and tryptophane accumulates in the tissue proteins. This failure is not due to a deficiency in the enzyme produced by *aa*, as the *Neurospora* hypothesis would suggest, since *aa* tissues contain an enzyme which can oxidize tryptophane to kynurenine. The effect of *aa* is rather to prevent enzyme and substrate, both of which are present in the mutant, from reacting together to produce kynurenine and hence brown pigment.

Coat Color in Rodents. The first characters in mammals to be studied genetically were the coat colors and patterns of the domesticated rodents: mouse, rat, guinea pig, and rabbit. Some of the interactions of the genes

affecting color have been described (p. 114). It has taken a much longer time to get some understanding of the developmental mechanisms by which the colors are reached, and the analysis is still far from complete. However, thanks to the work of Wright and others, the outlines of a physiological theory of color inheritance have begun to take form, based chiefly on studies on the guinea pig and the mouse.

The chief hair pigments are melanins, giving shades of yellow and sepia (including brown). In plants and invertebrates and probably in verte-

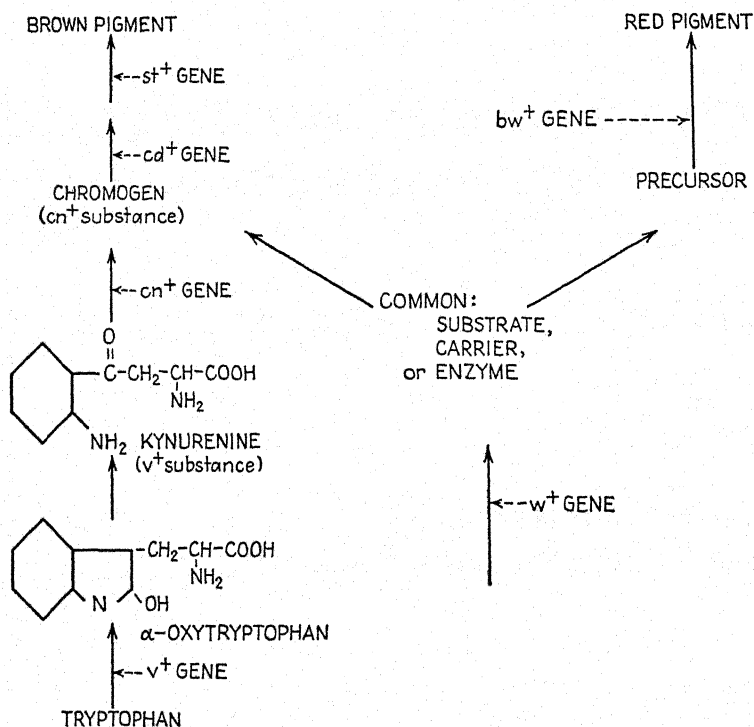


FIG. 183. The assumed reaction chain in the synthesis of brown and red eye pigments in insects. The wild-type genes (v^+ , cn^+) are assumed to facilitate specific reactions which fail to occur when the mutant allele is substituted (v , cn). (After Beadle.)

brates also, these pigments are produced by a chain of reactions in which a colorless chromogen, such as dihydroxyphenylalanine (dopa), a derivative of tyrosine, is oxidized to the colored substance, melanin, of which the exact chemical nature is still unknown. This is accomplished in vitro by an enzyme (dopaoxidase) but the exact nature of the enzymes in living individuals is unknown. According to Wright there are probably at least two enzymes, one concerned with black and brown (eumelanin) and another with yellow (phaeomelanin). Only the latter has been demonstrated.

Mutation at the albino locus interferes with production of both pigments, either completely in total albinos or partially in animals with intermediate alleles at the c^a locus (cf. p. 85). Yellow is more interfered with by these alleles than black or brown, and there is evidence of competition between the dark and yellow pigments for a limited amount of a common precursor. The genes for white spotting also interfere with both pigment systems in local areas, which are therefore colorless, probably because they influence the migration or survival of the cells (chromatophores) in which the pigment is formed. In the guinea pig there is a form of spotting of black and yellow known as tortoiseshell, which is due to an allele, e^p , intermediate between solid color or extension of black, E , and restriction of black to the eyes, e , leaving the coat yellow. With E , all areas reach a threshold at which eumelanin is formed; with e , all areas except the eyes are below this threshold so that only phaeomelanin (yellow) is formed; with e^p , the level is so near the threshold that differentiation may go in either direction, either to black-brown or to yellow, probably depending on local factors of which the chief is the level set by the white-spotting factors.

It is known that the melanin-bearing cells, the melanophores, arise in the early embryo from the neural crest of the developing neural tube and migrate to their final position, where they deliver the pigment granules for inclusion in the shaft of the growing hair. In birds, amphibia, and fishes, the movements of these cells, how long they survive, and what kind of pigment they produce are known to be controlled by genes, and the same is probably true in rodents and other mammals. Certain other genes in rodents affect only one or the other of the two chief hair pigments, such as the mutant gene p , which reduces melanin pigment in both hair and eyes but does not affect yellow, while mutation at the A (agouti) locus interferes with the entrance of melanic pigment into local regions of the hairs, leaving a longer or shorter yellow band in the agouti pattern or entirely yellow hairs with scattered melanin granules as in mutation A^u (cf. p. 98).

THE DEVELOPMENT OF SIZE DIFFERENCES

Hereditary differences in the size of the whole organism or of its parts usually depend upon many genes with similar and cumulative effects, so that it is seldom possible to trace the effects on size of single gene differences in development. On the other hand, such characters lend themselves readily to developmental study since they can be measured and described by the usual methods of morphology and embryology.

Differences in size are due to differences in the amount of growth, and thus the problem of the genetic control of size involves the genetic control of growth processes in general. Differences in the size to which related organisms grow or in the size of their parts may be determined by (1) differ-

ences in the initial size of the egg, the primordium, or bud from which its growth begins; (2) the rate at which it grows; (3) the length of time during which growth continues. Each of these is subject to genetic control.

Embryo Size. With the same rate and duration of growth, an embryo, or primordium, twice as large as another will attain double its size. This is especially well shown in differences in the size of fruits, as for example in tomatoes, in which large-fruited and small-fruited varieties differ already in the size of the first measurable primordium in the flower. The same is true in the races of cucurbits, in which genetic differences of the order of 500 times in final fruit volume are foreshadowed in the early primordia in the ovary.

Rate of Growth. These differences in early size must, of course, be due to still earlier differences in growth rate, and there is some evidence that such differences may obtain for a very short period in development. If one genotype differed from another by having just one more synchronous set of cell divisions than another, the final size of the two might well be markedly different. Castle and Gregory have indeed shown that as early as 40 hours after fertilization the embryos of a race of large rabbits are already larger than those of a smaller race, owing probably to differences in the rate of very early cell division. These in turn are probably related, according to Gregory, to the larger amounts of glutathione in the embryos of races of rabbits and fowls which attain large size as adults. The implication is that genes which cause more rapid early growth may do so by way of the metabolic steps which are stimulated by the sulfhydryl ion of glutathione.

Such differences in the early rate of growth as are implied by the above are probably of common occurrence, both in differentiating larger from smaller organisms and in causing differences in the sizes of the parts. Thus tall persons often differ from shorter ones in the speed of growth during the adolescent spurt of growth just before puberty, and this affects chiefly the length of the legs. "Creeper" fowls owe their short legs to a reduction in the general rate of growth at the time when the limb anlage in the embryo are growing most rapidly (p. 431). In many cases in plants, however, where the growth rates of large and small organs have been measured, they have been found to be remarkably similar. Thus a large pumpkin and a small gourd have essentially the same growth rate, their differences being due to differences in initial size or in duration of growth.

Duration of Growth. In general, larger animals and plants tend to have a longer growing period than smaller ones. Among annual plants, tall races are usually those which continue growing for a relatively long period before being checked by the development of flowers or some other physiological factor. Genes that affect the time of flowering or the position of the inflorescence may in this way influence the height of the plant. In beans,

a single gene determines the difference between plants with axillary flowers, which by growing continuously until checked by external conditions become the tall, climbing type of plants, and those in which growth in length is stopped by the terminal flower cluster, producing the short, bushy type of plant. Such genes probably produce their effects by way of plant hormones. This is known to be the case in the mutant form "lazy" in maize, which lies prostrate on the ground, and fails to grow upward by negative geotropism as in the normal plant. The mutant phenotype develops because the growth hormone, auxin, which in a normal plant placed on its side moves to the bottom side of the stem and stimulates growth there, causing the tip to turn upward, in a "lazy" plant has a uniform distribu-

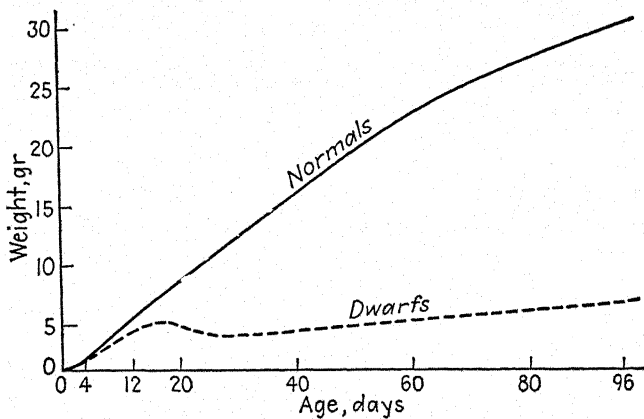


FIG. 184. Growth curves of average weight of normal and dwarf (*dw/dw*) mice. (After Francis.)

tion so that both sides of the stem grow with equal speed and no upturning occurs.

In animals, dwarfism in at least one case, is also determined by a single gene difference which operates by way of a hormone. Mice homozygous for the mutant gene *dw* stop growing when about two weeks of age and reach only about a third of the size of their normal litter mates, and although they are otherwise healthy, they are always sterile (Fig. 184). It has been shown by Smith and MacDowell that the dwarf gene interferes with the production of a growth hormone by the anterior lobe of the pituitary gland. When this hormone is supplied through the implantation of pituitary substance from normal mice or rats, the dwarfs resume growth and the males may become fertile. The normal allele of *dw* is thus shown to be concerned with the production of a substance which regulates the duration of growth.

The Cellular Basis of Size Differences. Differences in size between

individuals or races or between organs of the same individual usually involve differences in the size or the numbers of cells. In the case cited above of the races of rabbits differing in total size, Painter found no difference in cell size but a great difference in cell number. Large leaves and fruits likewise usually contain more rather than larger cells.

On the other hand the larger size of tetraploid plants, especially autotetraploids, is often associated with a cell volume about twice as great as in the diploid. Haploid plants, in the same way, are smaller in all their parts than diploids, and their cells have about half the volume of diploid cells. Such differences have been reported in *Datura* (Fig. 185), maize, tomato, and many other seed plants and are also conspicuous in the extensive polyploid series in mosses as studied by von Wettstein.

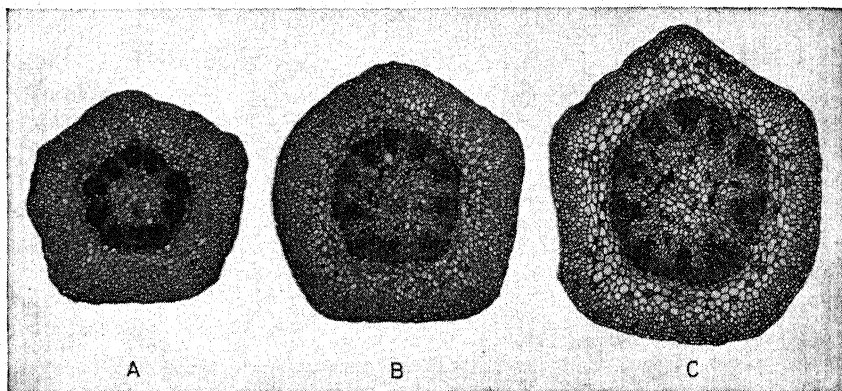


FIG. 185. Cross sections of flower stalks of haploid (A), diploid (B), and tetraploid (C) plants of *Datura*.

The only polyploid series in animals which has been thoroughly studied from this standpoint shows an interesting case of regulation in which total size accommodates itself to changes in cell number. As already described (p. 374) Fankhauser, by the use of low temperature, produced haploid, triploid, and tetraploid salamanders (Figs. 186–188) in which the volumes of nuclei and of cells were proportional to the number of chromosomes; yet the animals with larger cells were of normal size, indicating that growth had ceased after fewer cell divisions.

Cell size and body size may be affected by genetical differences other than polyploidy. In *Drosophila*, Dobzhansky found that each small bristle on the wing corresponds to one cell, so that by counting the bristles in a measured area it is possible to calculate the average size of the individual cells. In this way it was found that the reduced size of wings in the mutant "miniature" is due chiefly to decreased cell size. By comparing

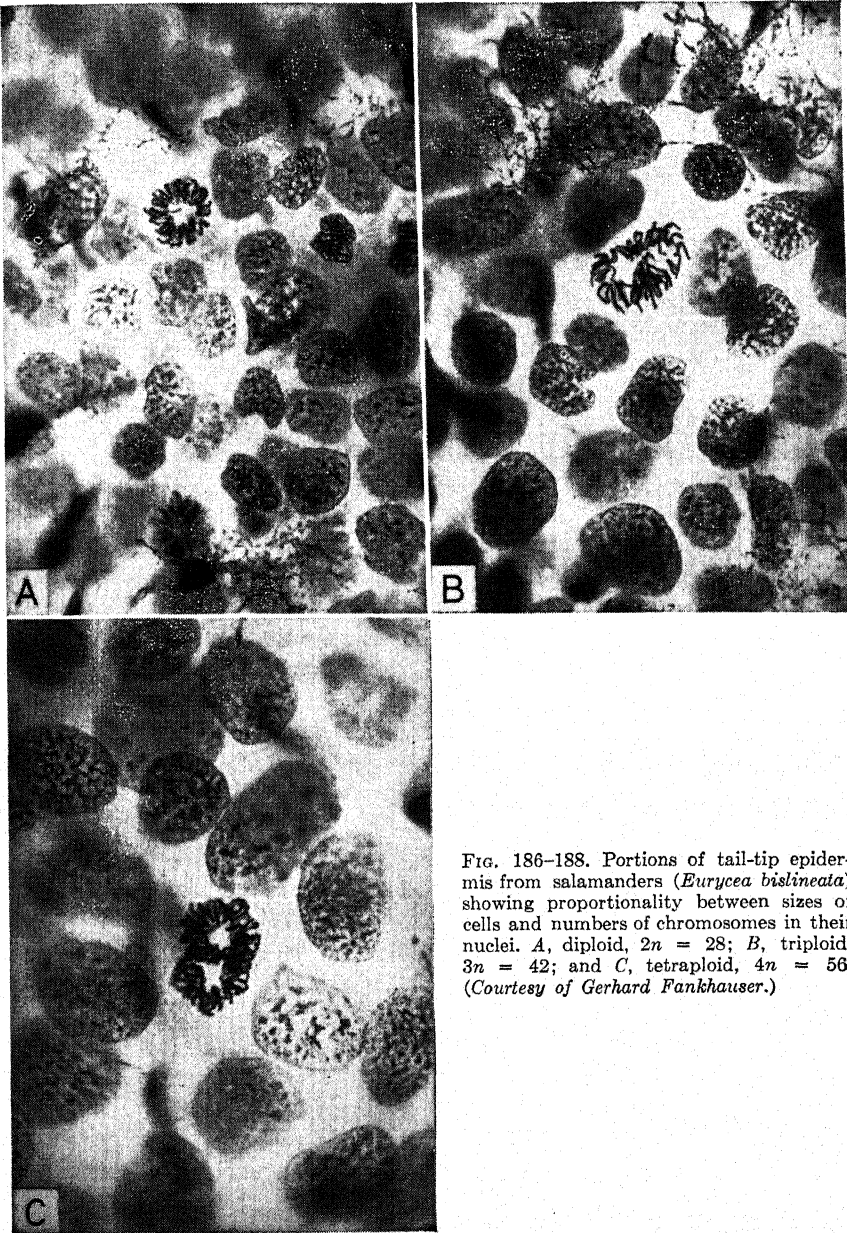


FIG. 186-188. Portions of tail-tip epidermis from salamanders (*Eurycea bislineata*) showing proportionality between sizes of cells and numbers of chromosomes in their nuclei. A, diploid, $2n = 28$; B, triploid, $3n = 42$; and C, tetraploid, $4n = 56$. (Courtesy of Gerhard Fankhauser.)

cell sizes in males, females, triploids, supermales, superfemales, and intersexes, among which there are marked differences in chromosome number and volume, it was found that cell size in general increases with increase in amount of chromatin. Such a relationship does not hold for all organisms, apparently, for Goldschmidt found that in races of *Lymantria* differing considerably in chromatin mass there were no significant differences in cell or body size.

Aside from these instances where cell size is related to chromosome number or volume there are many others, especially in plants, in which it seems to depend on ordinary genic differences. Thus among the many races of *Cucurbita pepo*, which differ greatly in fruit size, there are very considerable differences in cell volume, the cells of the fruit wall at maturity ranging from 300,000 cubic microns in some races to about twenty times that volume. Cucurbit fruits well illustrate the importance of cell size in plant development, for there is often an increase of about 10,000 times in cell volume between the tissues of the tiny ovary primordium and the tissues of the mature fruit. In extreme cases this difference may even reach 100,000 times. In the early stages of development, until about the time of flowering, cell multiplication occurs, and the number of cells is determined. Division now ceases, and cell expansion takes place. The extent of both cell division and cell expansion is important in determining fruit size. Both are clearly inherited, and they seem to be independent of one another genetically. The effects of single genes have as yet been shown in only a few such traits, since most of them seem to be governed by multiple genes.

THE DEVELOPMENT OF FORM DIFFERENCES

The possession of a specific form, both of the body as a whole and of its component parts, is one of the most distinctive features of organisms. These forms are clearly under the control of genes, and they provide some of the most familiar examples of Mendelian heredity. The single gene difference between spherical and disk fruits in squash; between spherical and pear-shaped fruits in tomato; between lobed and entire leaves in Japanese morning-glory; between single, rose, and pea combs in poultry; and between the many wing forms in *Drosophila* may be cited. The mechanism by which form differences are determined involves the development of form in general, and this in turn is closely related to the whole problem of organization and organic correlation, as to the control of which very little is known.

In the simplest cases, where the dimensional relationships of a single organ are involved, a developmental analysis is not difficult. Cucurbit fruits differing in shape have been measured from their earliest visible primordia

to maturity and the origin of these shape differences thus observed. The *relative* rates of growth of the various dimensions are often unequal and are found to be constant over long periods and therefore to produce a constantly changing form. Thus in the "Club" gourd, length constantly grows faster than width for most of its development, so that the shape changes from almost isodiametric in the smallest primordium to one that is about fifteen times as long as wide in the mature fruit. In other races, on the contrary, growth in width exceeds that in length by a constant amount. The ratio between the two dimensional growth rates does not change and may be

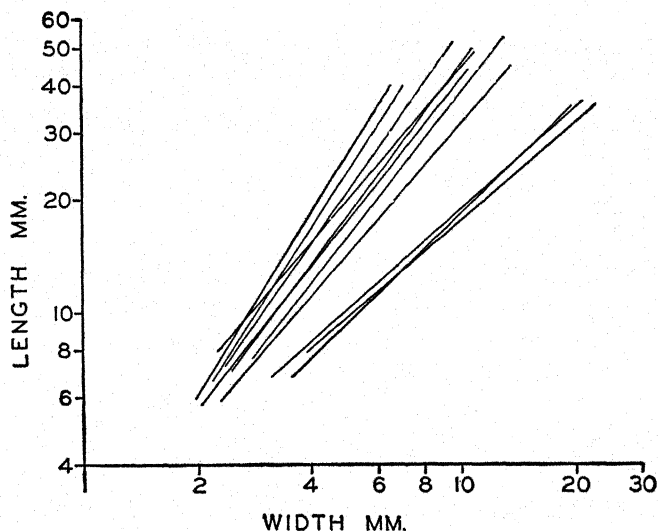


FIG. 189. Segregation of a single-gene difference in relative rate of growth of fruit length and width. An F_2 from a cross between two pure lines of *Lagenaria*, in one of which length grows relatively fast and in the other relatively slowly, in comparison to width. The eight plants at the left show the dominant growth ratio, the three at the right, the recessive. Both are plotted logarithmically.

expressed by a constant, α , the ratio of the two logarithmic growth rates to each other.¹ Experiment indicates that it is this ratio, rather than any particular ratio between dimensions, that is genically controlled (Fig. 189). The duration of the period of unequal growth rates may be brief, establishing a shape for the young ovary which then persists through later development when growth is uniform in all dimensions; or it may continue much longer. Such differences in dimensional growth rates occur pri-

¹ In the formula of simple allometry as stated by Huxley and Teissier, where $y = bx^\alpha$. In the present case, x and y are dimensions, b is the value of y when $x = 1$, and α is the growth ratio.

marily during the period of cell division and are related to constant differences in the plane in which the cells divide. This, in turn, is evidently one manifestation of cell polarity. The genic control of shape in such cases therefore seems to be exercised through a control of cell polarity. Less commonly, dimensional changes are associated with modification of cell shape during the period of cell expansion.

These relatively simple form changes arising from constant differences in relative dimensional growth rates are common throughout animals and plants. The genic mechanisms by which they are controlled are far from clear. Hormone action, differences in electric potential, and various other means for producing inequalities in growth have been suggested, but without proof.

More complex form differences involve changes, not in a single organ, but in the relative growth rates of two organs or parts, by which the general bodily pattern is altered. Here again it is commonly found that, although two such parts are growing at different rates, the ratio between these rates is constant, so that a constant and predictable change in form occurs during growth. This is the general phenomenon of allometry, to which attention has been particularly directed by Huxley, Teissier, and others. A well-known example is that of the crab, *Uca pugnax*, where the large claw grows faster than the rest of the body so that the ratio of claw to body changes as the animal grows. Here again the ratio of the two growth rates is constant. Many similar examples could be cited from animal and plant development. Allometric growth is merely the old problem of organic correlation expressed in more precise terms. These constant growth relationships are clearly inherited, but here, too, we have little information as to how genes control them except that the control is exercised over the whole system or body.

In the case of direction of coiling in the shells of snails (*cf.* p. 440), it has been shown that a form difference so striking as to reverse the usual symmetry of the organism may be due to a genic effect on a single cell division.

Genic Modification of Embryological Processes. Many genetically controlled differences are sharply localized and involve differences in the relative size, form and function of specific parts, such as the mutants with changed eye or wing shape in *Drosophila*, or the variant forms of legs, tail, feathers, or other parts in birds and mammals, or the variations in the size and form of leaf, flower, or fruit. Here the problem is to explain what seems to be a localized effect of a gene difference on one organ or part. Such cases can be studied by standard embryological methods and the difference traced to its first appearance. When this is done, it often turns out that the apparently localized effect is actually a secondary result of some more general change, such as (1) a modification of an over-all rate of growth or metabolism which finds one part of the organism in an especially

sensitive or plastic state or (2) a changed dependence or "organizer" relationship which particularly affects those parts whose fates are being determined; (3) changes in circulating substances like hormones which

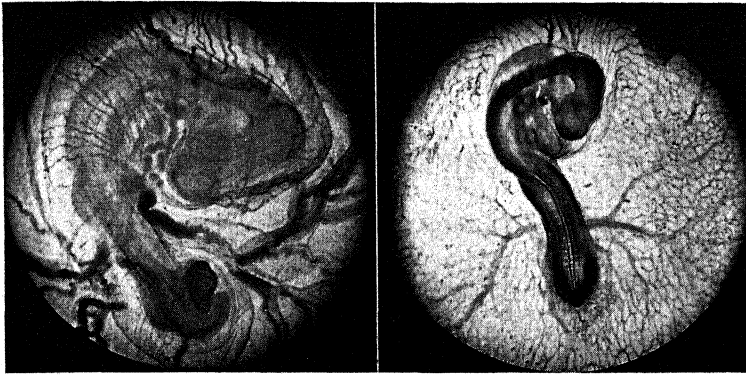


FIG. 190. Photographs at same magnification of embryos of normal chicken (left) and homozygous Creeper (right) at 72 hours of incubation to show delayed growth of the Creeper, which is about to die. (From Landauer.)

have local effects or (4) changes in the movements or multiplication of particular groups of cells which affect the character of an organ or tissue. Some illustrative examples of each of these will be briefly described.

General Metabolic Effects. The "Creeper" fowl with much shortened legs differs from the normal by a dominant mutation which is lethal when homozygous. Landauer has shown that this mutation has many effects on the skeleton and other parts and that the several effects are consequences of a retardation of growth which occurs in the early embryo. Embryos homozygous for this mutation die at about 72 hours of incubation (Fig. 190); but already at 36 hours they have fallen behind the normal in growth, and the anlage of the legs, which in the normal are growing rapidly at this time, fails to grow in the homozygotes. Rarely one of these lethal embryos lives until a later embryonic period; such animals are smaller than normal, have only rudiments of hind limbs, and have characteristic abnormalities of head, eyes, and other parts (Fig. 191). An embryological study of the parts reveals that they deviate from normal in the order of their normal growth potencies during the early period of retardation; that is to say, the parts which in the normal are growing most rapidly at that time are most retarded and abnormal in the homozygote.



FIG. 191. A homozygous Creeper embryo which has lived beyond the lethal period, showing marked changes in legs (only toes showing), wings (less reduced), head and eyes. (From Landauer.)

In the heterozygotes a similar relationship holds, the longer and more distal limb bones being most shortened. These differences in limb length and proportion are established in the early embryo, and thereafter the different bones grow at about the same relative rates so that the altered growth pattern appears as a result of the retardation suffered in the earlier period.

The effect of the Creeper mutation appears to be of a general, nonspecific character since Fell and Landauer induced the same effects by growing normal limb rudiments in culture media with abnormally low nutritive contents. David showed that, while most tissues of homozygotes had normal growth potencies when removed and grown in normal culture media, the heart tissue was defective and did not grow normally.

The parts of the embryo seem to respond to the lowered metabolism brought about by the mutation, in accordance with differences in the needs imposed upon them by their growth rates and their position in the developmental system. Thus a generalized change may have specific or localized effects, such as that in the circulatory system, and these, in turn, may have general effects on structures developing later, which suffer in different degrees from lack of nutrients or oxygen. The pattern of effects which follows very early modifications of development may thus be expected to be an alternation of general and specific effects.

"Organizer" Effects. In some cases the mutation disturbs some organic correlation or dependence of one developing part on another. An example of this is found in the so-called *T* mutations in the house mouse, which produce Brachyury, or short-tailed phenotypes, and Anury, or taillessness, together with many other effects on formative tissues. Here, as Chesley, Gluecksohn-Schoenheimer, and Dunn have shown, the shortness or absence of the tail is due to the absence of the posterior part of the notochord. The neural tube and somites in the normal embryo require some influence from the notochord for their normal development; when this is lacking, they undergo the first differentiation but later degenerate so that the tail distal to the end of the notochord is resorbed before birth (Fig. 192). Embryos homozygous for the Brachyury mutation *T* always die before birth, at the end of 10 days of development. In them the notochord is lacking, somites and neural tube are grossly abnormal, the posterior part of the body including hind limbs does not develop and there is no functional allantois or umbilical vessel and hence both the nutrition and excretion of the embryo are abnormal.

There is another mutation, t^0 , close to *T* and showing no crossing over with it, which in combination with *T* (Tt^0) produces taillessness. Such mice breed as a balanced lethal system (cf. p. 300). The homozygotes t^0t^0 die shortly after implantation, and produce no mesoderm or mesodermal

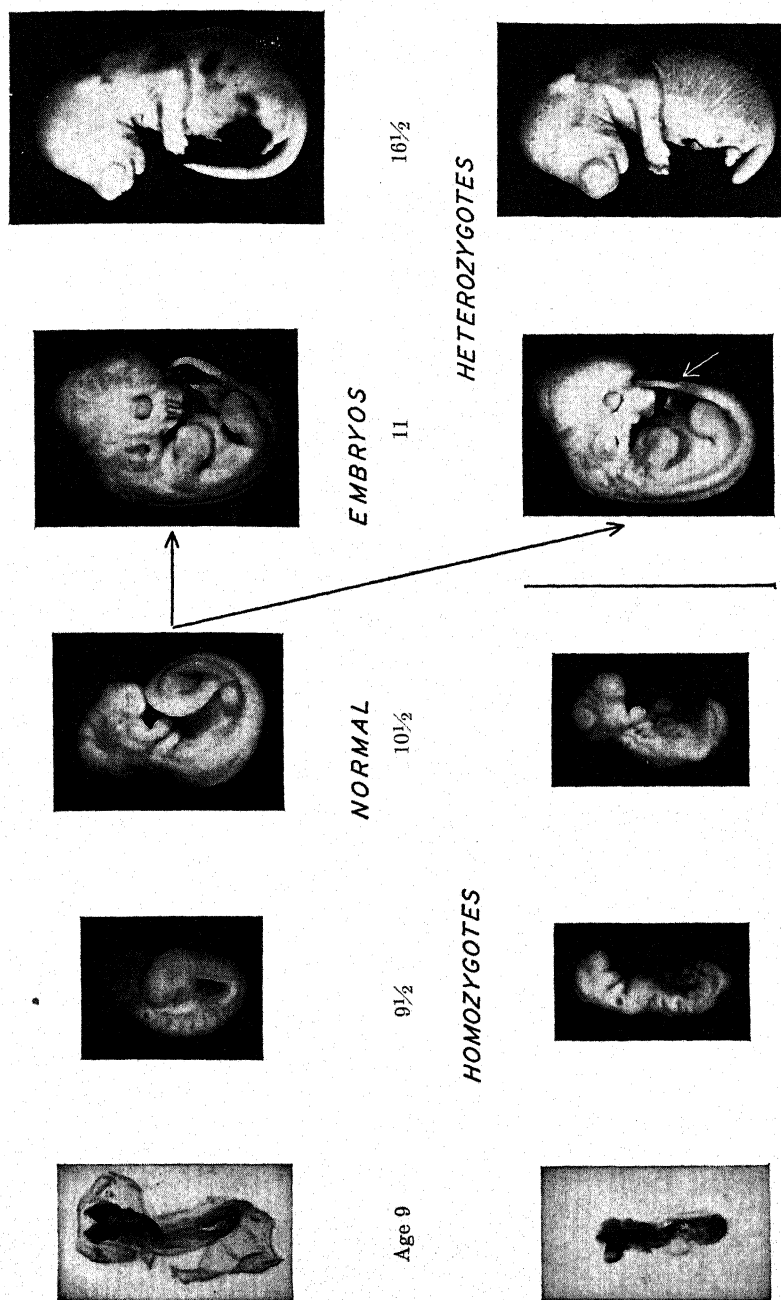


FIG. 192. The effects on development of a mutation in the house mouse. Above, from left, normal embryos of 9, 9½, 10½, 11, and 16½ days of development; below, litter mates of above, showing homozygotes at 9, 9½, and 10½ days and heterozygotes at 11 and 16½ days. The homozygote at 10½ days is shown just before death and lacks tail bud and hind limb buds. In the heterozygote at 11 days the constriction in the tail marks the end of the notochord; by 16½ days the end of the tail has been resorbed. (After Chesley.)

structures. This mutation appears to interfere at an earlier point than *T* with an inductive chain of events leading from mesoderm through notochord formation to organization of the skeletal and muscular systems. The chain itself, that is, the system of inductive relationships resulting in normal development, is thus shown to be controlled by genes.

In similar ways specific genes may determine which of two alternative paths of development may be realized. Thus in mice with the mutant gene flexed (*fl fl*) the cartilage between certain of the developing vertebrae of the tail differentiates not into the normal fibrous tissue of the intervertebral cushions or disks but into bone so that neighboring vertebrae are fused

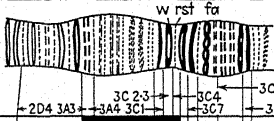
DEFICIENCY	CYTOLOGICAL EXTENT	EMBRYOLOGICAL EFFECTS		
		TISSUES AND ORGANS FROM ENDODERM MESODERM ECTODERM		
W 258-11		ABNORMAL GUT	INCOMPLETE MUSC	NORMAL
W 258-14		" "	" "	" "
W 258-45		" "	" "	" "
NOTCH-8		INCOMPLETE GUT	UNDIFFERENTIATED	GIANT NERVOUS SYST. SKIN REDUCED
N 264-38		" "	" "	" "
NOTCH-B		" "	" "	" "
N 264-19		" "	" "	" "
N 264-8	NO VISIBLE DEFICIENCY	" "	" "	" "
N 264-40	" " "	" "	" "	" "
N 264-47	" " "	" "	" "	" "
N 264-34 (T1,3L)	" " "	" "	" "	" "
N 264-53 (T1,2L)	NO VISIBLE DEFICIENCY	" "	" "	" "

FIG. 193. Relation between extent of chromosomal deficiency and effect on embryonic development of 12 mutations near the Notch region of the X chromosome of *Drosophila melanogaster*. Black indicates visible deficiency in the salivary-gland chromosomes of the bands included in the salivary map at top; crosshatched columns indicate genetical deficiency not visible in the chromosomes. Absence of facet locus produces the earliest effect on development; deficiency for white produces later effect. (After Poulson.)

together, often at an angle to each other, forming crooked parts or kinks. Whether bone or felted fibers arise depends, according to Kamenoff, on the speed of the preceding cell divisions, and this may be connected with the fact that the flexed gene produces also an embryonic anemia which lowers the oxidative capacity of the blood and probably the speed of growth.

In *Drosophila*, Poulson has studied the effects of a series of X-chromosome deficiencies, including the mutation Notch, on the differentiation of the early embryo. The degree to which differentiation proceeds depends on the X chromosome genes which are missing. When there is no X chromosome (nullo-X), development ceases after the first cleavage divisions when the nuclei fail to migrate to the periphery to form the blastoderm. Embryos with only a small deficiency in the Notch

region (*cf.* Fig. 193) undergo the first differentiations normally, but in them many more cells than normally differentiate into neural tissue, mesodermal derivatives are defective, and disorganization and death ensue about halfway through the embryonic period. Genotypes intermediate in amount of deficiency between these extremes show intermediate degrees of defectiveness. It is apparent that the missing genes are concerned with the organization and regulation of the cell movements and other processes of the early embryo.

Hormonal Effects. Another of the correlative systems in the higher animals and plants through which genes exert their effects on development is that concerned with hormonal coordination. Chemical substances produced in one part of the body circulate through it and produce specific effects on other parts which are sensitive to the particular hormone. Examples of this have been cited in connection with the pituitary dwarfism in the mouse and in man, the "lazy" mutation in maize, and the development of eye-color differences in *Drosophila* and *Ephestia*. Further understanding of the relation between genes and hormones has been obtained through the work of Danforth and others, who have studied the manner of determination of genetical variations in the plumage of birds. By exchanging transplants of skin between newly hatched chickens differing in sex and feather pattern and color it has been shown that, although the pattern of the feathers is determined by the genes in the follicle, its form is influenced by the hormones of the host. Thus skin transplanted from a barred Plymouth Rock female to a Rhode Island Red male produces barred feathers of the male type. In the dove, on the other hand, sex differences in the plumage are regulated entirely by the genes without reference to the hormones, and it appears that the effects of hormones depend upon the reactivity or threshold of the feather follicles. From this and other studies it appears that sex hormones in birds are essentially nonspecific substances to which tissues of different species or genotype may or may not develop a capacity to respond. Genic effects are therefore mediated in two different ways with respect to hormones, (1) by controlling the chemical constitution of a substance or of the cells which produce it and (2) by conditioning the cytoplasm of the responding cells and thus determining to which hormones they will respond.

Summary. The examples given above show that the differentiating characters of animals and plants acquire their final form by virtue of genic effects mediated through enzymes, antigens, organizer substances, and hormones and possibly through other channels yet to be discovered. A change in a gene (mutation) may lead to an increase or diminution in the amount of one of these effective substances. The most usual effect of a new mutation seems to be to reduce the quantity of some essential substance or reaction below the threshold level required for some develop-

mental process, since, in general, mutant genes are less efficient than those alleles which have been built into the species genotype by natural selection. These changes in the quantities of substances produced by or affected by the genes can seldom be directly demonstrated in the organism but generally have to be inferred from the altered quantity of a reaction and particularly from the changes in the rate of one process or of development as a whole which so frequently accompany mutant effects.

Although, as we shall see in the next chapter, it is not possible to determine as yet whether the first, or primary, effects of genes are of one kind only (such as the production of a specific antigen or enzyme) or of several kinds, it is already apparent that the secondary and later effects of gene action are both many and various and form parts of a great network of interactions of which the external effects on the characters were discussed in Chapter V.

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CHAPTER XVII

THE CYTOPLASM IN HEREDITY AND DEVELOPMENT

The question whether heredity was transmitted through the nucleus or through the cytoplasm at one time loomed large in biological discussions. In the decade following the rediscovery of Mendel's laws in 1900, few geneticists dared to believe that inheritance through genes was anything but a special case of heredity. Thus, Johannsen and some other pioneer geneticists were inclined to think that genes determine only so-called "superficial" traits, while "fundamental" traits of the organism are inherited through the cytoplasm. Advances in our knowledge of heredity however, have, left no reasonable doubt that genes in the chromosomes account for the transmission of most of the individual, racial, specific, and other traits which have been investigated. Only in a few cases, mostly in plants, evidence has been found that some traits are transmitted through the cytoplasm. Such traits are inherited usually in the female line only. A modern geneticist is led to ask, not whether the nucleus or the cytoplasm has an exclusive control over heredity and development, but rather how the genes and the cytoplasmic constituents interact.

For convenience in discussion, each set of nuclear genes is sometimes designated as a *genome*, while the totality of the heredity transmitted through the cytoplasm is referred to as the *plasmon*.

Maternal Inheritance. As a rule, the offspring of reciprocal crosses ($A \text{ } \varnothing \times B \text{ } \sigma$ and $B \text{ } \varnothing \times A \text{ } \sigma$) are alike. We know that the two sexes have equal ability to transmit heredity to the offspring because the male and female gametes carry similar complements of genes. In sex-linked inheritance the reciprocal crosses are, of course, not alike, but this exception is easily accounted for by the known behavior of sex chromosomes (see Chap. XV). Occasionally, however, reciprocal crosses are unlike because the offspring resemble in certain traits the female parent only, as though these traits are transmitted exclusively through the female gametes. Such *matroclinous* inheritance can arise from either one of two causes. First it may be due to the characters concerned being inherited through the cytoplasm (*cytoplasmic inheritance*). The amount of cytoplasm in a female gamete is in most organisms vastly greater than in a male gamete; hence the mother is more potent in the transmission of such characters than the father. Second, since the egg cell with its cytoplasm develops in the

mother's body under the influence of the maternal genes, certain characteristics of the embryo which arise from the egg may be likewise influenced more strongly by the genes of the mother than by those of the father. This *maternal effect* is due to *predetermination* of the egg cytoplasm by maternal genes.

We may consider maternal effects first. The simplest kind of such effects is a transmission in the cytoplasm of materials elaborated by the mother's genes. Thus, in *Ephestia* moths (cf. p. 418), crosses of $aa \text{ } \text{♀} \times Aa \text{ } \text{♂}$ individuals produce caterpillars only *half* of which show the darkening

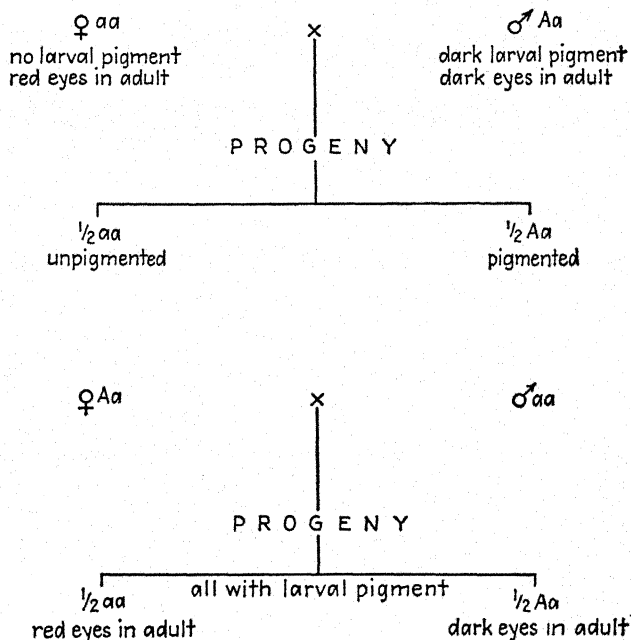


FIG. 194. Maternal effect of the gene *A* in reciprocal crosses in *Ephestia Kühniella*.

effect of the *A* gene, whereas in crosses $Aa \text{ } \text{♀} \times aa \text{ } \text{♂}$ *all* the caterpillars are at first dark because all eggs contain the pigment precursor, kynurenine, for which the gene *A* is responsible (Fig. 194). The effect of this gene wears off as the development proceeds, and the adults from these reciprocal crosses are alike. We are evidently dealing here with effects of nuclear genes which are stored in the egg cytoplasm to a greater extent than in the sperm, and hence it is unnecessary to postulate any cytoplasmic factors to account for such cases.

A very clear case of predetermination of traits by maternal genes has been

observed in the water snail *Limnaea*. Many species of snails are known in which the shell always coils to the right (dextral) and many others in which it coils to the left (sinistral). In a few species both dextral and sinistral individuals occur. In one of these (*Limnaea peregra*) dextrality appears to behave as a simple dominant to sinistrality, as shown by the breeding experiments of Boycott, Diver, and Garstang, as interpreted by Sturtevant. The character of the coiling, however, is determined not by the individual's own genes but by those of its mother. Some of these snails which are themselves phenotypical dextrals produce all sinistral offspring; and such individuals by appropriate genetic tests may be shown to be homozygous for the recessive sinistral gene. Their dextral character

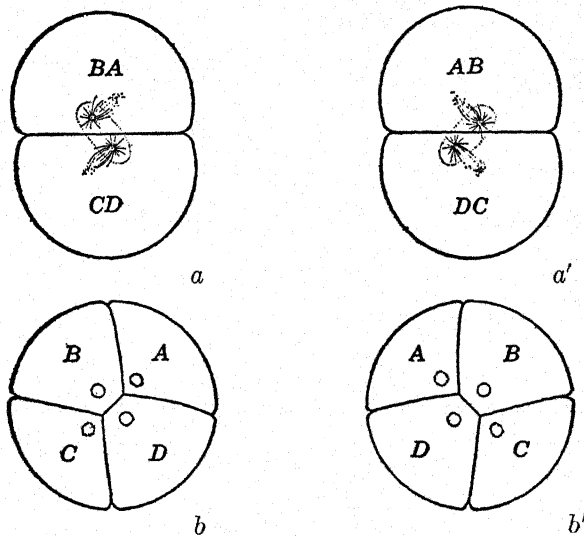


FIG. 196. Above, the first cleavage stage in a snail, showing position of spindles for next cleavage division in left-handed (sinistral) cleavage (*a*) and right-handed (dextral) cleavage (*a'*). Below, same after cleavage; *b*, sinistral type, *b'*, dextral type. (After Morgan.)

must have been determined by the presence of a dextral gene in their mothers (Fig. 195). This shows that it is not the "character" of the mother but her genes which impress upon the cytoplasm of all her eggs before maturation a certain type of pattern which finds expression during the early cleavage divisions of the egg. This phenomenon has been called "maternal determination."

The direction of coiling in snail's shells had previously been shown by Crampton, Conklin, and others to be determined by the orientation of the spindle at the second (possibly at the first) cleavage division. In the sinistral type the spindle is tipped toward the left of the median line; in the dextral type, toward the right (Fig. 196). This is in turn determined

by some relationship of the egg to the mother before maturation. This gene thus acts on the eggs in the ovary, influencing the direction of an early cell division and thus the type of asymmetry and the pattern of the future individual.

Both of the above cases and a number of others like them are due to effects of nuclear genes, which are expressed or stored in the egg cytoplasm to a greater extent than in the sperm. It is therefore unnecessary to postulate factors in the cytoplasm, or a plasmon, to account for them.

In other cases of maternal inheritance, changes induced by treatment are transmitted for several generations but eventually disappear. Thus Jollos produced modifications in protozoa by treatment with chemicals. These reappeared in the offspring in the absence of treatment for many generations, whence they were called persisting modifications (*Dauermodifikationen*). Later, Jollos and others produced such modifications by heat-treatment of *Drosophila* which were transmitted through the female line for several generations. It is apparent that in this case neither a gene nor a stable plasmon is involved but rather the transmission of some influence which fails to reproduce itself or to effect permanent or genic changes.

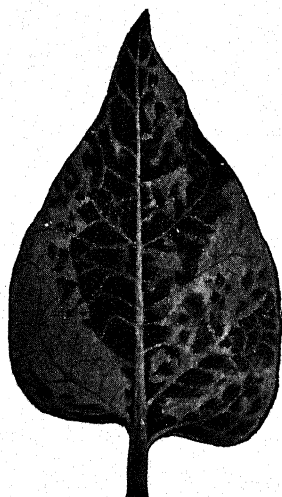


FIG. 197. Albomaculatus variegation; a leaf of a variegated four o'clock, *Mirabilis jalapa*. (After Correns.)

Transmission of a Plasmon. The best known cases of continuous transmission of stable elements through the cytoplasm concern plastid inheritance in plants. Correns and others, for example, studied the "albomaculatus" type of leaf variegation, in which the normal green tissue is irregularly spotted with patches of paler green or white. These may be

small or may include entire leaves or branches. This character occurs in a wide variety of plants, and its inheritance has been determined in more than 20 genera. Flowers on wholly green branches produce seed which grow into normal plants; flowers on variegated branches yield offspring of three kinds, green, white, and variegated in variable proportions; flowers from branches wholly white give progeny without chlorophyll; but in every case the source of the pollen has no influence on the offspring (Fig. 197). Inheritance is wholly maternal. Variegation seems clearly to be determined by agencies localized in the cytoplasm rather than in the chromosomes. A satisfactory explanation of the mechanism of inheritance for such a trait is available, however, since variegation is evidently the result of differences

in chloroplast development and since the primordia of these bodies, from which the plastids of the whole plant are ultimately derived, are present in the cytoplasm of the egg. For this system Renner has proposed the term *plastome*. In a few cases, as in *Pelargonium zonale*, the variegation, which appears as a colorless margin to the leaf, may be transmitted through both parents (though in an irregular fashion), perhaps because a little cytoplasm enters with the male nucleus. The situation is complicated by the fact that such plants are periclinal chimeras, with one or two layers of colorless cells surrounding a core of green.

The transmission of plastid primordia through the cytoplasm is good evidence of the existence of a stable system of self-perpetuating bodies outside of the nucleus. Nevertheless, in most cases, hereditary variations in plastids, resulting in albinism, striping, and other types of variegation, show normal Mendelian inheritance and are hence ascribable to gene mutation.

The question of the relation between self-reproducing bodies in the cytoplasm, such as plastids, and nuclear genes has recently been resolved in one clear case by Rhoades. He found that maize plants homozygous for a recessive gene *ij* (*iojap*) in chromosome VII are usually green- and white-striped, owing to the presence of some defective colorless plastids in the white parts. The cross of *Ij Ij* ♀ (normal green plant) by *ij ij* ♂ (striped plant) produces only normal green *Ij ij* plants; but when *ij ij* plants, as females, are fertilized with pollen from normal green *Ij Ij* ones, F_1 consists of mixtures of normal green, white-striped, and all white (albino = non-viable) seedlings. When striped F_1 plants, *Ij ij*, as females are test-crossed by normal green *Ij Ij*, they produce in some cases only green, in others green-striped and white, and from some ears only white seedlings. Since these backcross progenies all consist of *Ij Ij* and *Ij ij* plants in equal numbers, this means that in the all-white or all-striped progenies the abnormal character of the plastids appears in plants which lack the *ij* gene. Rhoades has given other evidence for the conclusion that, in *ij ij* plants, normal plastids may become permanently changed (mutated) to a deficient colorless condition and that this is perpetuated by plastid reproduction in the absence of the gene that induced the mutation. The abnormal plastids are then passed on through the ovules; they may happen to constitute a part of the plastids (giving striped progeny) or all of the plastids in one ovule or a sector or whole ear (in which case all-white progenies are produced). It is evident that here an important relationship is revealed by which changes in a self-reproducing plasmon are induced by an element of the genome. A somewhat similar situation has been revealed in *Paramecium* by Sonneborn and his associates and will be discussed later (p. 446).

Other Cases of Cytoplasmic Transmission. Several cases have been studied in which maternal inheritance occurs in traits which are not concerned with plastids, and these provide a more difficult problem. Among these is the case of male sterility in maize described by Rhoades. This character, which consists in the abortion of much or all of the pollen (though not the ovules) is transmitted solely through the mother and never by the pollen. Since gene markers are known for all the 10 chromosomes of maize, Rhoades was able to replace each of the chromosomes of the male-sterile race with one from normal stock. Male sterility was found not to belong to any of the 10 linkage groups thus tested and seems to be controlled by some agency in the cytoplasm independent of the chromosomes.

A somewhat more complex situation occurs in the genus *Epilobium*, a member of the Onagraceae closely related to *Oenothera*, which has been studied intensively by Lehmann, Michaelis, Renner, and others. Reciprocal crosses between *Epilobium hirsutum* and the markedly different *E. roseum* are very dissimilar. Where *hirsutum* is the female parent, the offspring are nearly sterile and have anthers and petals which are much reduced in size. Where *roseum* is the female parent, there is little sterility and the floral parts are well developed. There are also reciprocal differences in the size of the plant and of its vegetative organs. By repeated back-crossing of the F_1 (*roseum* ♀ × *hirsutum* ♂) with *hirsutum* male, Michaelis produced a type in which the cytoplasm was derived from *roseum* but the chromosomal complement was presumably now entirely from *hirsutum*. When this was crossed reciprocally with pure *hirsutum*, similar differences were observed as when pure *roseum* and pure *hirsutum* were crossed, indicating that these differences were due to the cytoplasm, since the genes were now presumably identical. Renner and Michaelis regard the evidence from *Epilobium* as strongly indicative of an hereditary vehicle in the cytoplasm. Lehmann and his students explain the differences in reciprocal crosses in this genus as due to the production of specific changes in the cytoplasm by different gene combinations or to differences in reaction of a given nucleus in different cytoplasm.

Somewhat different in type are the extensive studies of von Wettstein on mosses of the family Funariaceae. He observed no differences in reciprocal crosses between varieties of *Funaria hygrometrica*, the characters segregating in normal Mendelian fashion. When the two species *F. hygrometrica* and *F. mediterranea* were crossed, however, marked differences between the reciprocal crosses appeared. For most traits the segregating gametophyte offspring tended to resemble the maternal parent, the paternal types often failing to appear. A few characters showed normal segregation. When the two genera *Funaria* and *Physcomitrium* were crossed, the offspring were all similar to or identical with the female parent.

Many of the spores were sterile. Von Wettstein interprets these results as due to the failure of genomes similar to that of the male parent to survive and function in cytoplasm derived from a different source. This conclusion is supported by evidence from polyploid races produced by regeneration of gametophytes from sporophyte tissue. By this means it was possible to introduce as many as three sets of paternal chromosomes from one genus into the eggs of the other genus, but even with this preponderance of the male genomes, inheritance was still entirely maternal. These facts show convincingly that there are marked developmental incompatibilities between genes and cytoplasm and that, when the two are derived from different sources, the cytoplasm may prevent the genes from acting.

Evidence as to cytoplasmic inheritance can be derived from quite another source, through the study of cases of *merogony*, where the egg cytoplasm comes from one parent and the nucleus (sperm) from the other so that their effects can be compared. The classic experiments in this field were performed by Boveri, who fertilized enucleated eggs of one species of sea urchin with sperm from a markedly different one. Although development did not persist beyond the pluteus stage, the merogonous embryos seemed to resemble the species from which the sperm was derived, indicating that the nucleus controlled development even when surrounded by cytoplasm from a very different source. Boveri's results are open to serious question, however, since there is evidence that the nuclear material was not entirely removed from the egg, and since the resemblance to the male parent might well be explained on simple genetic grounds. In other cases, undoubted merogonous embryos did not develop far enough to establish whether nucleus or cytoplasm is in control. The work of Hadorn, however, indicates that for certain traits the male nucleus may be without effect in some merogonous tissues. Two species of Triton (*T. palmatus* and *T. cristatus*) differ clearly in the character of the epidermis. Hadorn fertilized the egg of *palmatus* with the sperm of *cristatus* and succeeded in removing the *palmatus* nucleus before nuclear fusion. The embryo (thus haploid) develops only to the blastula stage; but a portion of the presumptive epidermis, if grafted to a normal larva of another species, *T. alpestris*, maintains its identity and develops to the adult state. Here it resembles the epidermis of the parent *palmatus*, which contributed the cytoplasm; rather than the parent *cristatus*, which contributed the nucleus. For this trait, at least, the cytoplasm rather than the nucleus seems to have the decisive effect, a result quite the opposite from Boveri's.

Extrachromosomal Transmission in Animals. Several cases of extrachromosomal transmission have been established in animals. In *Drosophila*, l'Heritier and Teissier found a true breeding strain which differed

sharply from others in its high sensitivity to carbon dioxide. Reciprocal crosses between this and normal strains gave different results, sensitive mothers always having sensitive offspring, of which the females again transmit sensitivity during repeated outcrosses with nonsensitive males. Sensitive males may, however, transmit the peculiarity to a few of their offspring only, and of these some of the sensitive females may transmit it to some of their progeny. By replacing each of the chromosomes of the sensitive stock by homologues from a normal resistant stock the sensitive character was shown to be transmitted outside of the chromosomes and to be associated with a heat-labile substance, sigma, which is transmitted primarily through the egg cytoplasm but which can be separated from the animals and used to induce sensitivity in non-sensitive eggs by implantation of normal ovaries into sensitive females. Since sigma is self-perpetuating, it is assumed to be in the nature of a virus.

In outcrossing mice from a long inbred strain in which nearly all females developed a particular type of mammary cancer, Bittner found that cancer susceptibility was maternally transmitted. Moreover, by using females from the high-cancer strain as foster mothers for newborn mice from non-cancer strains and normal mothers as nurses for young from the high-cancer strain it was shown that the substance which induces cancer susceptibility is transmitted through the mother's milk, whence it has become known as the "milk factor." Recent evidence suggests that it is probably a virus, but whether it can reproduce itself indefinitely apart from a certain genetic constitution of the host is not yet known.

Interaction of Nuclear and Cytoplasmic Heredity in Infusoria. Sonneborn and his associates have discovered and described remarkable instances of cytoplasmic heredity in the infusorian *Paramecium aurelia*. Certain strains of this species, known as "killer" strains, secrete into the water in which they live a substance, paramecin, which injures and kills individuals of "sensitive" strains of the same species. Infusoria may reproduce for a long time asexually, by simple fission, forming genotypically uniform clones. Killer individuals always give killer clones and sensitive individuals, sensitive clones. In sexual reproduction, infusoria unite, or conjugate, in pairs. Their nuclei undergo meiotic divisions; some of the resulting nuclei degenerate, but two nuclei remain in each mated individual. One of these nuclei remains (Fig. 167), while the other migrates into the body of the mate, where it fuses with the stationary nucleus. The mated individuals then separate, each now carrying a diploid nucleus, which is a product of the union of two nuclei coming from different individuals. By appropriate procedures, individuals of killer clones may be crossed to sensitive ones. Observations on the offspring of such crosses have shown that killers carry

a dominant gene, K , while sensitive individuals are usually homozygous for the recessive allele, k .

When killers, KK , conjugate with nonkillers, kk , the exconjugants are, of course, all heterozygous Kk and should, accordingly, be killers. However, if the conjugation lasts for a very short time only, the results shown in Fig. 198 are observed. That is, a killer clone and a nonkiller clone are produced, although both have the same genotype, Kk . A more prolonged conjugation, which permits not only the exchange of nuclei but also some mixing of the cytoplasms of the mated individuals, results, however, in production of killer clones only. These facts are explained by assuming that the property of being a killer is due to the presence not only of the nuclear gene K but, in addition, of a cytoplasmic factor, kappa. Kappa is transmitted in vegetative reproduction through the cytoplasm which the daughter individuals inherit from their mothers and in conjugation if parts of the cytoplasms of the mates were exchanged. The presence of the gene K in the nucleus is not, however, sufficient to initiate the production of kappa in the cytoplasm. Thus a Kk individual may be either a killer or a sensitive individual. On the other hand, kappa cannot be maintained in the cytoplasm for a long time unless at least one gene K is present in the nucleus; kk individuals may inherit kappa in the cytoplasm, but they lose it after some cell divisions.

Units like kappa which undergo self-reproduction in the cytoplasm rather than in the nucleus, and transmit characters from cell to cell or individual to individual, are known as *plasmagenes*.

Preer has found that certain killer clones of *Paramecium* can be converted into sensitives by making the infusoria undergo very rapid fissions, during which the production of kappa particles cannot keep pace with the cell divisions, and the substance is finally lost entirely. It has also been possible to estimate the number of kappa particles in a killer individual and most recently to identify and count these particles under the microscope as tiny bodies which appear to contain desoxyribose nucleic acid. This system of plasmagenes transmitted in the cytoplasm is a true plasmon,

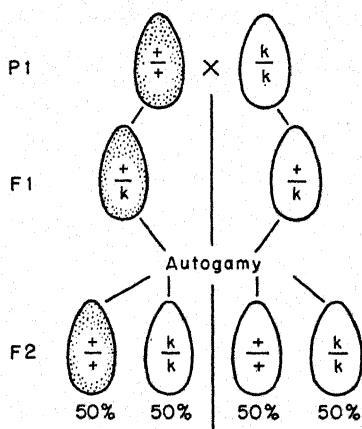


FIG. 198. Inheritance of the "killer" character in *Paramecium aurelia*, variety 4, when the killer gene K , is transmitted but not the killer cytoplasm. (After Sonneborn.)

although its permanence and stability depend on the relation between its rate of multiplication and that of cell nuclei and of the cells themselves. It resembles in this respect the milk factor and the sigma substance in carbon dioxide-sensitive *Drosophila*. All may turn out to be viruses which depend for their maintenance, if not for their origin, on a specific gene or genotype. Further discussion of the relation of such gene products to gene effects will be found in the next chapter.

For a full discussion of these and similar cases, the student is referred to the recent review of Caspari (1948), in which it is concluded that, while there is no longer reason to doubt that a plasmon exists, there appears to be no serious conflict between this type of transmission and the normal transmission and action of nuclear genes.

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CHAPTER XVIII

GENE ACTION AND THE NATURE OF THE GENE

Recent studies, especially those on hereditary effects transmitted through the cytoplasm, both between the generations and within the individual, have strengthened the view that the control of heredity is vested primarily in genes, which are discrete, self-reproducing parts of the cellular organization. The problem now is to understand how genes produce their effects on development. The identification of first substances, or gene products, formed by genes inside the cells; elucidation of the mechanisms of interaction of these substances; analysis of the kinds of changes that occur in genes and in their primary products when the genes mutate—these are fundamental procedures which must be undertaken in order to clarify the nature of the gene and of the way it reproduces itself from other materials present in the cell. As has been pointed out frequently in Chapter XI, the problem of self-reproduction of the gene is perhaps the most fundamental one of all, for it is the property of self-reproduction which enables the gene to maintain its basic integrity, upon which the continuity of life depends, and still to vary its effects, permitting progressive change and adaptation, which constitute the process of evolution.

One of the first questions that must be faced is to what extent the gene is an independent unit of structure and function within the chromosome and in what measure this unit may be integrated in and subservient to an organization of a higher order. The first genetic maps portrayed the chromosome as a succession of gene loci, scattered, apparently at random, without regard to their function in development. The view of the genetic chromosome as primarily a string of beadlike genes seems indeed to find its cytological counterpart in the succession of chromomeres in the prophase chromosomes (Chaps. VIII and X). The correspondence between the stainable disks in the chromosomes of salivary-gland cells in flies and the genetic linkage maps of chromosomes lent further support to the idea of the discreteness of genes and of successive loci. This view, however, was brought into question by the discovery of so-called *position effects*, which show that the function of a gene in development depends not only upon the intrinsic properties of that gene alone but also upon what genes lie next to it in the chromosome.

Position Effects. The first conclusively proved case of position effect concerns the mutation Bar eye* in *Drosophila melanogaster* (Fig. 199). This has long been known as a dominant located at 57.0 on the genetic map of the X chromosome (Fig. 98, p. 227). Its chief effect is to reduce the

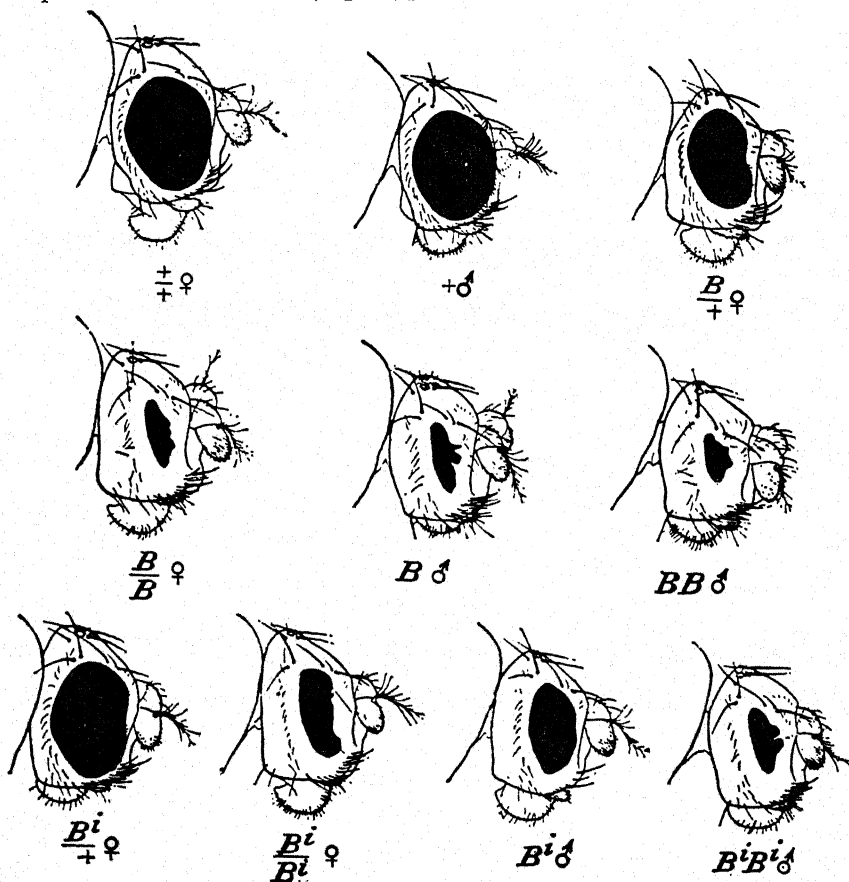


FIG. 199. Drawings of the head and right compound eye of members of the Bar series of phenotypes in *Drosophila melanogaster*. $\frac{B}{B}$ indicates a Bar mutation in both X chromosomes; BB , two Bar mutations in the same chromosome. B^i is a less extreme allele of B which also shows the position effect phenomenon. (After Morgan.)

number of facets in the compound eye, leaving a more or less narrow band, or "bar," of ommatidia. Zeleny found that in cultures homozygous for Bar there occur "mutations" back to wild-type, or normal, eye and to a more extreme type with very narrow eyes, called ultra-Bar. The frequency of these mutations is rather high, one reversion to normal appearing in about 1,500 offspring, and one ultra-Bar in about 2,000 flies. The wild

type obtained by reversion breeds true, but ultra-Bar reverts to Bar and to wild type with appreciable frequencies.

The nature of these peculiar mutations at the Bar "gene" was first clarified by Sturtevant and subsequently by Bridges and by Muller, Prokofieva-Belgovskaya, and Kossikov. The Bar chromosome proved to contain a duplication for a short section, consisting, as seen in salivary-gland cells, of about six disks (Fig. 200). The ultra-Bar chromosome contains this section in triplicate and the normal chromosome in single dose.

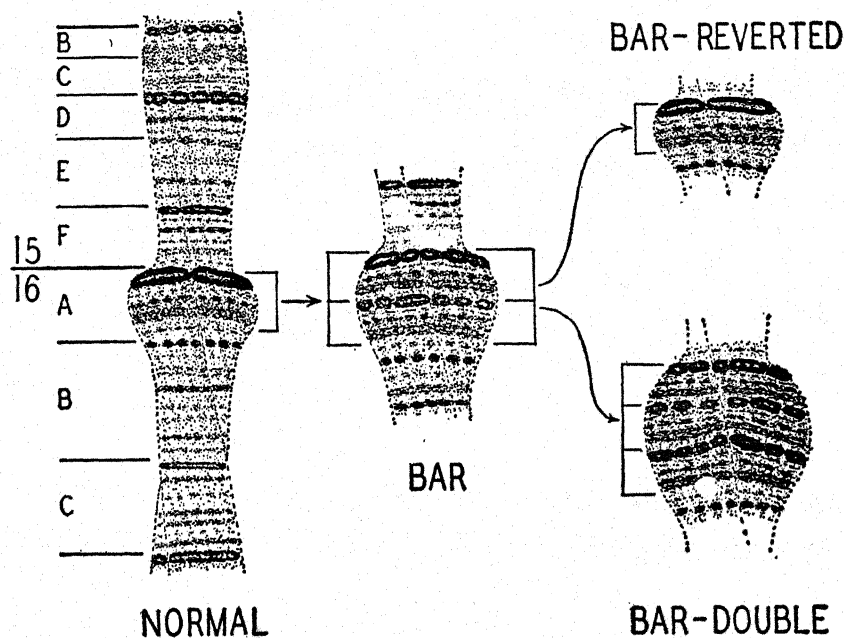


FIG. 200. The Bar region in salivary chromosome I, showing duplication of bands in Bar $\frac{B}{B}$, and Double-bar $\frac{BB}{BB'}$. (From Bridges.)

If this region of the chromosome be symbolized by letters ABCDEFGH, then the normal chromosome is ABCDEFGH, Bar is ABCDEFGBCDEFGH, and ultra-Bar is ABCDEFGBCDEFGBCDEFGH. The original change which produced the mutation Bar from normal was, then, a duplication of a short section of the chromosome. The relatively frequent changes from Bar to ultra-Bar and back to normal and from ultra-Bar to Bar and to normal involve no alteration in the genes as such. They occur because the chromosomes which contain duplications may undergo crossing over in several different ways, illustrated in Fig. 201. As a result of this cross-

ing over, chromosomes are formed which contain more or fewer Bar sections than did the original chromosomes. For example, crossing over between two Bar chromosomes, each containing the Bar section in dupli-

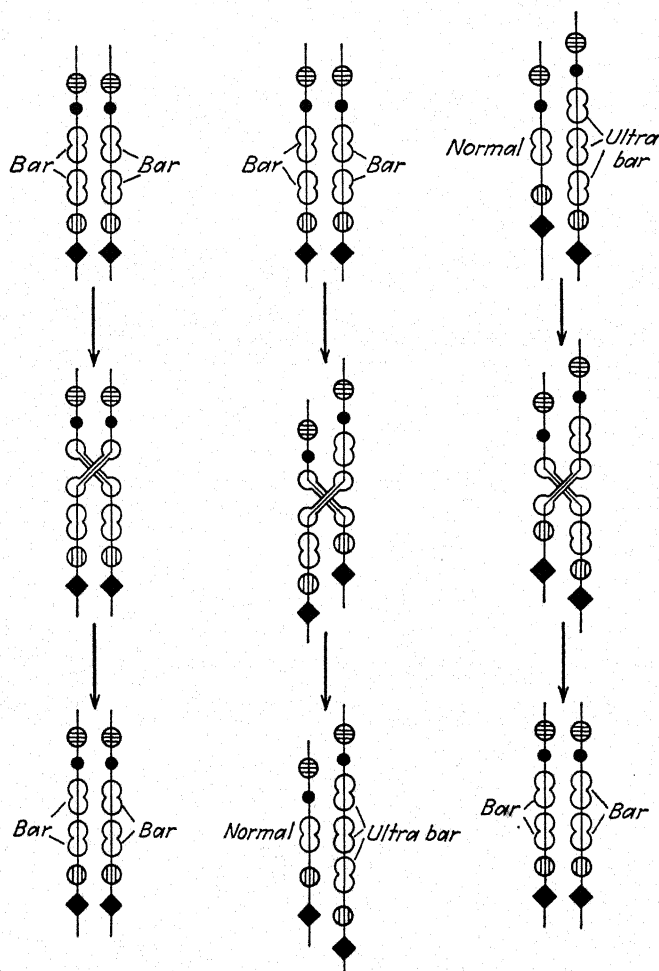


FIG. 201. Crossing over in Bar duplications, in *Drosophila melanogaster*. Left vertical column, crossing over between two Bar chromosomes leading to no changes in the Bar region; middle column, crossing over between two Bar chromosomes giving rise to a normal (non-Bar) and an ultra-Bar chromosome; right column, crossing over between a normal and an ultra-Bar chromosome giving rise to two Bar chromosomes.

cate, may give rise to chromosomes with a single Bar section (normal, or wild type) and with three Bar sections (ultra-Bar).

It follows from the above analysis that a fly homozygous for Bar (that is, carrying two X chromosomes, each with the "mutant" Bar) must have

four Bar sections, two in each chromosome. A fly heterozygous for normal and for ultra-Bar must also carry four Bar sections, one in the normal and three in the ultra-Bar chromosome. Since these flies carry the same sets of genes, they are expected to be alike in phenotype. But, as discovered by Sturtevant, the homozygous Bar flies have larger eyes (68.1 ± 1.1 ommatidia) than the heterozygous normal/ultra-Bar flies ($45.4 \pm .2$ ommatidia). This difference being quite significant statistically, it can be concluded, following Sturtevant, that juxtaposition of Bar sections in the same chromosome produces a stronger effect on the developing eye than the same sections would produce if placed in different chromosomes. In fact, it is probable that the difference between normal and Bar is also due to position effect, in other words, that the original mutation which gave rise to Bar from normal involved no change in the genes themselves but only change in their functioning due to alteration in their position. Indeed, in the normal chromosome, ABCDEFGH, the genes B and G have, respectively, the genes A and H as neighbors; in a Bar chromosome, ABCDEFGBCDEFGH, the genes B and G are neighbors; in an ultra-Bar chromosome there are two BG associations. The whole Bar story is explained very simply if one supposes that the association of the genes B and G causes a reduction of the number of facets in the compound eyes.

Since the discovery of position effect in Bar, other cases have been found in *Drosophila* by Dubinin and others, while Catcheside found at least one case in a plant, *Oenothera*. In *Drosophila*, translocations and inversions obtained by mutation are very often lethal in homozygous condition. Since translocation and inversion homozygotes (Fig. 107, p. 247) have the same genes as normal flies do, only differently arranged in the chromosomes, the lethality or other phenotypic effects produced by homozygosis for translocations and inversions may be due to position effects. An alternative hypothesis, namely, that the lethality is caused by destruction or injury of the genes coincident with the chromosome breakage, is, however, difficult to exclude, and consequently the position-effect hypothesis cannot be regarded as proved in these cases. Such a proof has, however, been given by Dubinin for some other changes associated with chromosomal aberrations. If a fourth chromosome of *Drosophila melanogaster* containing a normal dominant allele of the gene *cubitus interruptus* or if a third chromosome containing the normal dominant allele of the gene *hairy* is broken close to the loci of these genes, the normal alleles lose their dominance. Suppose, then, that we have a translocation in which the third chromosome is broken near *hairy* and the fourth near *cubitus interruptus*, and suppose that, by means of appropriate crosses, we make individuals heterozygous for such a translocation heterozygous also for the recessive genes *hairy* and *cubitus interruptus*, the recessives lying in the normal, unbroken chromosomes. Such heterozygotes show the effects of *hairy* and of *cubitus in-*

terruptus in their phenotype, as if the normal alleles of these genes contained in the translocated chromosomes had undergone a "mutation" which deprived them of their normal dominance. Dubinin showed that these normal alleles regain their original dominance if, by crossing over, they are removed from the translocated chromosomes and replaced in chromosomes with the normal gene order. This evidence of reversibility of the changes in the alleles of hairy shows that the loss of dominance in translocations was not caused by mutations but by changes in the associations between genes.

Just why a change in position of a gene within a chromosome should affect the functioning of that gene is as yet not known. Haldane has suggested that position effects are due to changes in the relationships of the chemical products which the genes send forth into the nucleus; the results of interaction between these immediate gene products may quite conceivably depend upon the distance between their sources. Thus, if the gene order ABCDE is changed to ADCBE, the substances produced by the genes A and B and the genes D and E, which in the original chromosome were being liberated by neighboring genes, are generated at points relatively far apart in the cell nucleus. Conversely, the genes A and D and the genes B and E become neighbors, and their products can interact immediately after their appearance in the neighborhood of the genes. Stern and Ephrussi prefer different kinds of explanations, based on the assumption of deformation of the protein molecules composing the genes in chromosomes which suffer changes in the gene arrangement. However that may be, the existence of position effects shows that a chromosome is not merely a string of independent genes but a system in which the spatial arrangement of parts is important. It may be said that not only the genes but also their arrangement in the chromosomes are controlled by natural selection in the process of evolution. On the other hand, the discovery of position effects has by no means undermined the validity of the gene theory. A chromosome does consist of genes, which are separable by crossing over, by mutation, and by chromosome breakage.

Relations of Gene Alleles. Since we learn about the existence of a gene when we can observe two strains or varieties which differ in some trait which undergoes segregation in hybrids, that is, which carry different alleles of a gene, the first question to be asked about gene function concerns the relations of dominant and recessive, or of normal and mutant alleles. Soon after the rediscovery of Mendel's laws, it was assumed that the recessive, or mutant, conditions usually represent absences of some positive contributions which the dominant, or normal, alleles make in the development of the organism. In its original crude form, this "presence-absence theory" did not survive the discovery of multiple alleles, which proved,

that a gene can exist in more than just two alternative, present or absent, states. When it was also discovered that recessive mutant alleles may sometimes mutate back to the dominant, or "normal," state, it became clear that not all mutations represent losses of genes. On the other hand, a good deal of evidence has accumulated in favor of the idea that most recessive alleles represent at least partial losses of gene activity, in other words, that recessives do what their dominant alleles also do but that the recessives do less of it.

When a gene in a chromosome is destroyed, we have a chromosome with a deficiency (see Chap. X). In diploid organisms deficiencies are rarely viable in homozygous condition, but when they are viable, deficiency homozygotes often resemble the homozygous forms of some recessive alleles known to be in the deficient part of the chromosome. For example, in *Drosophila melanogaster*, flies homozygous for a deficiency for the locus of the gene yellow, which lies in a normal X chromosome at the extreme left end, show a yellow body color characteristic of homozygotes for the recessive mutant allele yellow. Since the recessive allele has the same effect on the developing organism as does the destruction of the gene, it is tempting to assume that the recessive allele represents a complete or partial inactivation of the function of the gene in question. McClintock has recently obtained several small deficiencies in maize chromosomes, which in homozygous condition copy the effects of recessive mutants.

In a few cases it has been possible to study additions (duplications) of recessive genes in an otherwise diploid organism. Now, let us assume, as a working hypothesis, that two doses of a recessive allele, in a recessive homozygote, produce less of some substance or product than two or even one dose of the corresponding dominant allele (in a dominant homozygote or heterozygote). If this hypothesis is true, one may expect that individuals which carry three, four, or more doses of the recessive allele will more and more resemble the carriers of the dominant allele. Stern has tested this hypothesis on the recessive mutant gene "bobbed" in *Drosophila*, which causes shortening of the bristles on the body of the fly. The answer obtained is quite clear: Flies with three or four bobbed alleles, obtained by using duplications for the chromosome section which carries bobbed, have bristles about as long as normal flies and much longer than do flies homozygous for bobbed (two bobbed alleles) (Fig. 202). It looks as though each bobbed gene contributed its bit to some bristle-forming reaction, and four bobbed genes make about the same contribution as one normal (dominant) allele. On the other hand, some other genes behave in a way opposite to bobbed, or show no effects in duplications.

On the basis of experiments of this type, Muller has suggested the following classifications of the developmental effects of mutant genes:

1. *Hypomorphs*, those like bobbed, with a weaker effect than the ancestral, or normal, alleles.

2. *Amorphs*, those which, like yellow, seem to have no effect on development. Probably most gene changes, but by no means all, result in production of recessive hypomorphic or amorphic alleles.

3. *Hypermorphs*, which produce more of some substance or effect than the ancestral allele, such as a normal allele arising by mutation from a hypomorph.

4. *Neomorphs*, having a new type of effect, not found in the original type, such as Bar eye in *Drosophila*.

5. *Antimorphs*, which inhibit the effects of a normal, or ancestral, gene, such as the dominant white allele in poultry (p. 110).

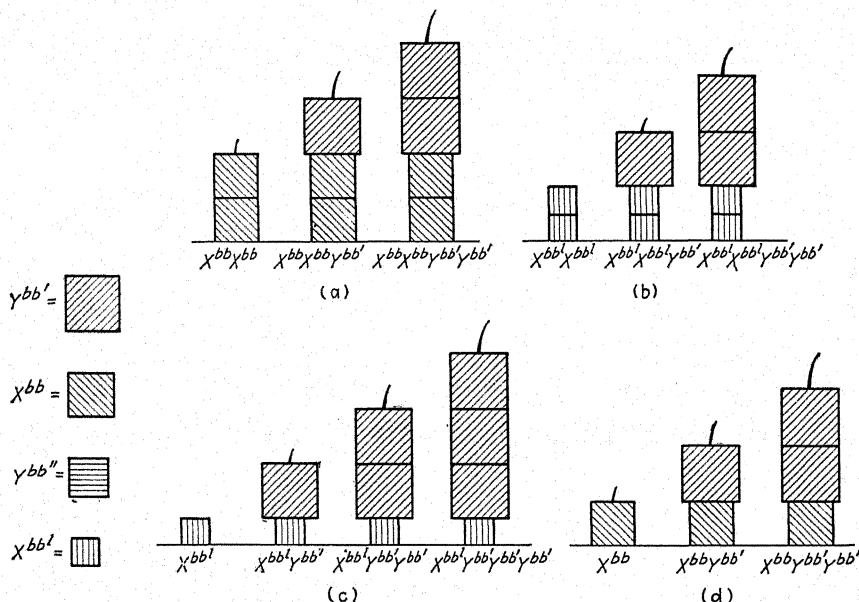


FIG. 202. Effects of alleles of the mutant gene bobbed (*bb*) on bristle length in *Drosophila melanogaster*. Relative effects of each allele at left; upper right (a and b) additive effects in females; lower right (c and d) additive effects in males. (After Stern.)

Dominance. As we have seen, the first interpretation of dominance as due to presence and of recessiveness as due to absence of a gene cannot be sustained. It may nevertheless still be true that more active alleles tend to be dominant over less active ones. But dominance cannot be satisfactorily explained by considering relations only between alleles of a single gene, because dominance is often affected by genes at other loci. The whole genotype of the organism is a product of natural selection in the

evolutionary history, and dominance is just one of the aspects of this historic development.

Members of a series of multiple alleles often show a quantitative seriation in their effects. The white series of eye-color alleles in *Drosophila* and the albino series of coat- and eye-color alleles in rodents are examples of this. In such series the most dominant member is often found in the wild representatives of the species (wild type), while the recessive members are mainly of mutant origin and occur chiefly under domestication or in laboratories and only rarely as ill-adapted aberrations in natural populations. These recessive alleles act, then, as hypomorphs and the most recessive one of all as an amorph. If all members of an allelic series show such graded effects, it is tempting to suppose that the gene in question is concerned with the production of just one substance or reaction, such as the eye-color hormone in *Drosophila*, but that different alleles produce different quantities of this substance. It must, however, be kept in mind that when we compare organisms carrying different alleles, such as *WW* for the normal and *ww* for the white eye color in *Drosophila*, all we can learn is the effect of the *difference* between the action of the allele *W* and the allele *w*, while both alleles may produce many other effects which are similar in both of them and therefore escape detection by usual genetic methods. To find out the sum total of effects of a gene, we must evidently compare organisms which carry this gene with others which do not carry it at all (deficiency). And it is a very suggestive fact that most homozygous deficiencies are lethal, even deficiencies for genes whose mutant alleles produce quite innocuous changes, such as making the coloration of some part of the body lighter or darker. The sum total of effects of a gene may, then, be much greater than comparison of effects of its alleles would suggest.

Primary Effects of Genes. The most important property of a gene is that it reproduces itself, that is, forms a copy of itself from materials present in the cell. Furthermore, genes may form and give off substances which influence specific reactions, first within the cell and subsequently elsewhere in the organism. These two properties or functions of genes, that of autocatalysis and of heterocatalysis, may prove to be two aspects of the same process: the specific gene products given off in the cell may be merely by-products of the reactions of gene synthesis.

However that may be, the substances through which the genes influence physiological processes in the cell must involve specific enzymes. Early in the development of genetics the attractive hypothesis was made that the gene either was itself an enzyme or produced an enzyme. In its modern form, this hypothesis as stated by Beadle supposes that a given enzyme will usually have its final specificity set by one and only one gene. The evidence for this one-to-one relationship between a gene and a single step in

a synthesis has been reviewed on pages 412 to 423. While it is true that in several cases mutations in individual genes have been shown to be responsible for failure of specific enzyme reactions (p. 413), there is as yet no evidence that the enzyme concerned is the immediate product of the gene.

On the other hand, there is some evidence that a gene acts directly to yield a product perhaps by transferring to it some portion of itself during gene reproduction. The production of specific immune substances has been assumed to occur in this way. In at least one case, a vitamin appears also to have been produced more or less directly by a gene. This may be inferred from the results of Mangelsdorf and Fraps. They took advantage of the fact that the difference between yellow and white endosperm in maize is determined by a pair of genes $Y=y$ and that Y is also responsible for the production of vitamin A, a yellow carotenoid. Because of the triploid nature of the endosperm in maize, it is possible to obtain, by proper crosses, maize seeds with endosperm of composition yyy , yyY , yYY , and YYY or with 0, 1, 2, and 3 yellow genes, respectively. Seeds of these four sorts, fed to rats according to a standard technique for estimating vitamin A units, proved to contain approximately 0, 2.25, 5.00, and 7.50 units of vitamin A per gram, while chemical determination of the carotenoids showed the same seriation of values. The amount of vitamin A was thus directly proportional to the number of Y genes, which makes it likely that Y is directly responsible for the formation of vitamin A and makes it unlikely that Y itself acts as an enzyme, since such direct proportionality is not a usual feature of enzyme action.

Since in so many instances genes have been shown to control the rates of reactions through specific enzymes, it may be that the gene confers specificity on proteins and converts them into enzymes by adding a particular prosthetic group which may be a vitamin. In the same way, a protein may be given an antigenic property by having added to it, by a gene, a smaller molecular grouping such as a hapten.

In any case, we must suppose gene action to be initiated by the production of a specific substance which has the same specific property as the gene because it was either a duplicate set free when the gene reproduced itself or a partial copy set free into the nucleus and thence into the cytoplasm. The former may be the source of the plasmagenes discussed earlier (p. 444).

Gene Reproduction. A diploid organism arises from a single cell with two representatives of each gene locus. In the course of its development each cell gets a replica of this original gene complement, which consequently must reproduce itself many times. This is one of the chief synthetic activities of growth, and it apparently involves a remarkable process by which one gene makes an exact copy of itself, utilizing and selecting from the food materials available to all the genes just those which are necessary

to reproduce its own specific properties. How this is accomplished is unknown, but the analogy between gene duplication and crystal multiplication has been repeatedly pointed out. This has taken on new meaning with the discovery that virus nucleoproteins, which have a power of specific self-duplication similar to that of genes, may be crystallized. It is true that, once obtained in crystal form, the virus protein can no longer produce copies of itself; it can reproduce only within a living cell which provides the necessary environmental conditions. There are, however, other close resemblances between viruses and genes, such as inactivation of viruses by the same agents (X rays and ultraviolet light) which change or destroy genes, and changes in specificity, which have now been shown to be due to mutation.

The facts of gene mutation (p. 303), which at bottom is due to imperfect copying of an ancestral gene by a descendant, suggest that the gene is a stable molecular configuration, which can impress its specificity on nongenic materials, so that copies may be struck off like coins from a die. The materials for the coin must always be assembled directly upon the die. This may be represented visibly by the pairing and attraction between homologous chromosomes, locus for locus, which is especially marked in the somatic pairing of chromosomes and in the close apposition of homologues in the salivary chromosomes of *Diptera*. But when excess energy from outside the molecular group (the die gene) such as an ionization or a photon is absorbed, the molecular pattern may be slightly changed. This new pattern may be copied and may thus become the new stable condition of the mutant gene. Often, of course, the damage to the die is so great that no copy can any longer be made, and this results in a gene deficiency.

Gene Number and Size. The discovery that genes are material particles located in chromosomes has raised many questions about the numbers, dimensions, and other attributes of genes. In the present state of our knowledge, no reliable answers to these questions are available, and some rough estimates that have been made should be treated merely as more or less plausible hypotheses that may be useful in further work.

As we have seen in Chapter X, some investigators suppose that there exists a one-to-one relationship between genes on one hand and some microscopically visible structures on the other, such as chromomeres at the pachytene stage of the meiotic prophase or disks in the salivary-gland chromosomes in flies. Counts of such structures indicate numbers of chromomeres of about 2,000 to 2,500 in a lily plant (Belling) and of about 5,000 to 6,000 in the salivary-gland chromosomes in *Drosophila* (Bridges). However, it is quite possible that some chromomeres contain more than a single gene, so that these counts are more likely to prove underestimates than overestimates.

In genetically well-known organisms, like *Drosophila* or maize, most of the mutations that are now being found in experiments are not new but are rather repetitions or recurrences of mutations that have already been observed in the past. This, of course, means that there exist only a limited number of genes that can produce mutations. If one assumes that all of these genes are equally likely to mutate, the frequency of repeated mutation would permit calculation of the number of genes. Such calculations give estimates of the same order of magnitude as those arrived at by chromomere counts. However, some genes are known to be more mutable than others, and this makes the calculations err on the side of underestimation.

Other estimates which give rough ideas of the gene size as well as number are arrived at by physical methods. One of these methods measures the gene as a target which gives rise to mutations when hit by X-ray bombardment. The numbers of X-ray quanta absorbed in a given volume of organic matter being known and the numbers of mutations produced thereby being recorded, estimates of target sizes may be made. It may be noted that estimates of sizes of virus particles made by this method agree fairly well with more direct measurements. The size of a gene target has been estimated by Catcheside as being some 2 to 9 millimicrons (10^{-6} mm.). This is most likely a minimum estimate, since the sensitive target may well be smaller than the gene and is less likely to be larger. An upper limit of gene size has been calculated by Muller by dividing the total volume of chromosomes by the estimated gene number. This assumes, of course that all chromosome material is genic. Measured by this method, genes would have sizes within the range of smaller viruses and of medium- to large-sized protein molecules (see Fig. 101, p. 240).

The only estimate of the gene number in a haploid-chromosome set in man is of the order of 30,000 to 40,000 and is based on frequencies of naturally occurring mutations. Little confidence can, however, be given even to this order of magnitude.

Genes and Viruses. The similarities between genes and viruses in several fundamental properties have been recognized for many years. Both are capable of self-duplication but only within living cells, viruses as parasites, genes as parts of chromosomes. The basic chemical structure in each is a combination of nucleic acid and protein. Some viruses can be obtained in crystalline form, and the crystals, when placed within the living cells of a susceptible host, give rise again to self-perpetuating bodies with activities and abilities very like those of genes. One of the most important properties shared by viruses and genes is the ability to mutate to new stable forms, producing new alleles in the case of the gene, new strains in the case of virus. Similar agents such as X rays and ultraviolet induce such changes in both genes and viruses. Different strains of the same virus are known

which consist of different nucleoproteins, leading to the speculation that gene mutation also involves substitution of one nucleoprotein for another.

Viruses, in fact, behave as though they were parasitic, subcellular organisms consisting only of genic material. If this proves to be true, then the gaps between organic molecules, genes, viruses, and microorganisms might be bridged. It is interesting to speculate that the first living substance to appear on earth might have been something like a gene or like a nonparasitic, free-living virus. Such a primordial virus may have been either autotrophic, that is, able to reproduce its like from inorganic materials, or it may have subsisted on more complex molecules, resembling the present organic ones, which may have been formed by chance on the earth's surface and persisted since there were no organisms to utilize them as food and thus to break them down. However that may have been, mutations in the primitive viruses altered their physiological properties, giving rise both to autotrophic organisms and to heterotrophic ones, which utilize, as all animals do, organic materials elaborated by other living beings. Present-day viruses would then be looked upon as having lost their ability to live outside of other organisms and to have become parasites, or symbionts, of living cells which satisfy their food requirements.

What Is a Gene? The gene, which was first identified as the unit of hereditary transmission, has, by further research, gained an even more fundamental place in the organization of living material. It may be regarded as a unit of structure and a unit of function as well, responsible for originating the synthetic activities of the cell which result in growth and differentiation in higher forms. It has come to occupy also a key position in evolution, not only because, by gaining the ability to duplicate itself and to mutate, it provided an essential early step in the origin of living matter, but because the permanent evolutionary changes in those living organisms which have been carefully studied appear to rest ultimately on particular kinds and distributions of genes.

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APPENDIX

EXPERIMENTS IN PLANT-HYBRIDISATION¹

BY GREGOR MENDEL

(Read at the Meetings of the 8th February and 8th March, 1865.)

Introductory Remarks

EXPERIENCE of artificial fertilisation, such as is effected with ornamental plants in order to obtain new variations in colour, has led to the experiments which will here be discussed. The striking regularity with which the same hybrid forms always reappeared whenever fertilisation took place between the same species induced further experiments to be undertaken, the object of which was to follow up the developments of the hybrids in their progeny.

To this object numerous careful observers, such as Kölreuter, Gärtner, Herbert, Lecoq, Wichura and others, have devoted a part of their lives with inexhaustible perseverance. Gärtner especially, in his work "*Die Bastarderzeugung im Pflanzenreiche*" (The Production of Hybrids in the Vegetable Kingdom), has recorded very valuable observations; and quite recently Wichura published the results of some profound investigations into the hybrids of the Willow. That, so far, no generally applicable law governing the formation and development of hybrids has been successfully formulated can hardly be wondered at by anyone who is acquainted with the extent of the task, and can appreciate the difficulties with which experiments of this class have to contend. A final decision can only be arrived at when we shall have before us the results of detailed experiments made on plants belonging to the most diverse orders.

Those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations.²

¹ This translation was made by the Royal Horticultural Society of London, and is reprinted, by permission of the Council of the Society, with footnotes added and minor changes suggested by Professor W. Bateson, enclosed within []. The original paper was published in the *Verhandlungen naturforschender Verein in Brunn, Abhandlungen*, iv. 1865, which appeared in 1866.

² [It is to the clear conception of these three primary necessities that the whole success of Mendel's work is due. So far as I know this conception was absolutely new in his day.]

It requires indeed some courage to undertake a labour of such far-reaching extent; this appears, however, to be the only right way by which we can finally reach the solution of a question the importance of which cannot be overestimated in connection with the history of the evolution of organic forms.

The paper now presented records the results of such a detailed experiment. This experiment was practically confined to a small plant group, and is now, after eight years' pursuit, concluded in all essentials. Whether the plan upon which separate experiments were conducted and carried out was the best suited to attain the desired end is left to the friendly decision of the reader.

Selection of the Experimental Plants

The value and utility of any experiment are determined by the fitness of the material to the purpose for which it is used, and thus in the case before us it cannot be immaterial what plants are subjected to experiment and in what manner such experiments are conducted.

The selection of the plant group which shall serve for experiments of this kind must be made with all possible care if it be desired to avoid from the outset every risk of questionable results.

The experimental plants must necessarily—

1. Possess constant differentiating characters.
2. The hybrids of such plants must, during the flowering period, be protected from the influence of all foreign pollen, or be easily capable of such protection.

The hybrids and their offspring should suffer no marked disturbance in their fertility in the successive generations.

Accidental impregnation by foreign pollen, if it occurred during the experiments and were not recognized, would lead to entirely erroneous conclusions. Reduced fertility or entire sterility of certain forms, such as occurs in the offspring of many hybrids, would render the experiments very difficult or entirely frustrate them. In order to discover the relations in which the hybrid forms stand towards each other and also towards their progenitors it appears to be necessary that all members of the series developed in each successive generation should be, *without exception*, subjected to observation.

At the very outset special attention was devoted to the *Leguminosae* on account of their peculiar floral structure. Experiments which were made with several members of this family led to the result that the genus *Pisum* was found to possess the necessary qualifications.

Some thoroughly distinct forms of this genus possess characters which are constant, and easily and certainly recognizable, and when their hybrids are mutually crossed they yield perfectly fertile progeny. Furthermore, a disturbance through foreign pollen cannot easily occur, since the fertilising organs are closely packed inside the keel and the anther bursts within the bud, so that the stigma becomes covered with pollen even before the flower opens. This circumstance is of especial importance. As additional advantages worth mentioning, there may be cited the easy culture of these plants in the open ground and in pots, and also their relatively short period of growth. Artificial fertilisation is certainly a somewhat elaborate process, but nearly always succeeds. For this purpose the bud is opened

before it is perfectly developed, the keel is removed, and each stamen carefully extracted by means of forceps, after which the stigma can at once be dusted over with the foreign pollen.

In all, thirty-four more or less distinct varieties of peas were obtained from several seedsmen and subjected to a two years' trial. In the case of one variety there were noticed, among a larger number of plants all alike, a few forms which were markedly different. These, however, did not vary in the following year, and agreed entirely with another variety obtained from the same seedsman; the seeds were therefore doubtless merely accidentally mixed. All the other varieties yielded perfectly constant and similar offspring; at any rate, no essential difference was observed during two trial years. For fertilisation twenty-two of these were selected and cultivated during the whole period of the experiments. They remained constant without any exception.

Their systematic classification is difficult and uncertain. If we adopt the strictest definition of a species, according to which only those individuals belong to a species which under precisely the same circumstances display precisely similar characters, no two of these varieties could be referred to one species. According to the opinion of experts, however, the majority belong to the species *Pisum sativum*; while the rest are regarded and classed, some as sub-species of *P. sativum*, and some as independent species, such as *P. quadratum*, *P. saccharatum*, and *P. umbellatum*. The positions, however, which may be assigned to them in a classificatory system are quite immaterial for the purposes of the experiments in question. It has so far been found to be just as impossible to draw a sharp line between the hybrids of species and varieties as between species and varieties themselves.

Division and Arrangement of the Experiments

If two plants which differ constantly in one or several characters be crossed, numerous experiments have demonstrated that the common characters are transmitted unchanged to the hybrids and their progeny; but each pair of differentiating characters, on the other hand, unite in the hybrid to form a new character, which in the progeny of the hybrid is usually variable. The object of the experiment was to observe these variations in the case of each pair of differentiating characters, and to deduce the law according to which they appear in the successive generations. The experiment resolves itself therefore into just as many separate experiments as there are constantly differentiating characters presented in the experimental plants.

The various forms of peas selected for crossing showed differences in the length and colour of the stem; in the size and form of the leaves; in the position, colour, and size of the flowers; in the length of the flower stalk; in the colour, form, and size of the pods; in the form and size of the seeds; and in the colour of the seed-coats and of the albumen [cotyledons]. Some of the characters noted do not permit of a sharp and certain separation, since the difference is of a "more or less" nature, which is often difficult to define. Such characters could not be utilised for the separate experiments; these could only be applied to characters which stand out clearly and definitely in the plants. Lastly, the result must show whether they, in their entirety observe a regular behaviour in their hybrid unions, and whether from

these facts any conclusion can be come to regarding those characters which possess a subordinate significance in the type.

The characters which were selected for experiment relate:

1. To the *difference in the form of the ripe seeds*. These are either round or roundish, the depressions, if any, occur on the surface, being always only shallow; or they are irregularly angular and deeply wrinkled (*P. quadratum*).

2. To the *difference in the colour of the seed albumen* (endosperm).¹ The albumen of the ripe seeds is either pale yellow, bright yellow and orange coloured, or it possesses a more or less intense green tint. This difference of colour is easily seen in the seeds as [= if] their coats are transparent.

3. To the *difference in the colour of the seed-coat*. This is either white, with which character white flowers are constantly correlated; or it is grey, grey-brown, leather-brown, with or without violet spotting, in which case the colour of the standards is violet, that of the wings purple, and the stem in the axils of the leaves is of a reddish tint. The grey seed-coats become dark brown in boiling water.

4. To the *difference in the form of the ripe pods*. These are either simply inflated, not contracted in places; or they are deeply constricted between the seeds and more or less wrinkled (*P. saccharatum*).

5. To the *difference in the colour of the unripe pods*. They are either light to dark green, or vividly yellow, in which colouring the stalks, leaf-veins, and calyx participate.²

6. To the *difference in the position of the flowers*. They are either axial, that is, distributed along the main stem; or they are terminal, that is, bunched at the top of the stem and arranged almost in a false umbel; in this case the upper part of the stem is more or less widened in section (*P. umbellatum*).³

7. To the *difference in the length of the stem*. The length of the stem⁴ is very various in some forms; it is, however, a constant character for each, in so far that healthy plants, grown in the same soil, are only subject to unimportant variations in this character.

In experiments with this character, in order to be able to discriminate with certainty, the long axis of 6 to 7 ft. was always crossed with the short one of $\frac{3}{4}$ ft. to $1\frac{1}{2}$ ft.

¹ [Mendel uses the terms "albumen" and "endosperm" somewhat loosely to denote the cotyledons, containing food-material, within the seed.]

² One species possesses a beautifully brownish-red coloured pod, which when ripening turns to violet and blue. Trials with this character were only begun last year. [Of these further experiments it seems no account was published. Correns has since worked with such a variety.]

³ [This is often called the mummy pea. It shows slight fasciation. The form I know has white standard and salmon-red wings.]

⁴ [In my account of these experiments (*R.H.S. Journal*, vol. xxv, p. 54) I misunderstood this paragraph and took "axis" to mean the *floral* axis, instead of the main axis of the plant. The unit of measurement, being indicated in the original by a dash ('), I carelessly took to have been an *inch*, but the translation here given is evidently correct.]

Each two of the differentiating characters enumerated above were united by cross-fertilisation. There were made for the

1st trial 60 fertilisations on 15 plants.

2nd	"	58	"	"	10	"
3rd	"	35	"	"	10	"
4th	"	40	"	"	10	"
5th	"	23	"	"	5	"
6th	"	34	"	"	10	"
7th	"	37	"	"	10	"

From a larger number of plants of the same variety only the most vigorous were chosen for fertilisation. Weakly plants always afford uncertain results, because even in the first generation of hybrids, and still more so in the subsequent ones, many of the offspring either entirely fail to flower or only form a few and inferior seeds.

Furthermore, in all the experiments reciprocal crossings were effected in such a way that each of the two varieties which in one set of fertilisation served as seed-bearer in the other set was used as the pollen plant.

The plants were grown in garden beds, a few also in pots, and were maintained in their naturally upright position by means of sticks, branches of trees, and strings stretched between. For each experiment a number of pot plants were placed during the blooming period in a greenhouse, to serve as control plants for the main experiment in the open as regards possible disturbance by insects. Among the insects¹ which visit peas the beetle *Bruchus pisi* might be detrimental to the experiments should it appear in numbers. The female of this species is known to lay the eggs in the flower, and in so doing opens the keel; upon the tarsi of one specimen, which was caught in a flower, some pollen grains could clearly be seen under a lens. Mention must also be made of a circumstance which possibly might lead to the introduction of foreign pollen. It occurs, for instance, in some rare cases that certain parts of an otherwise quite normally developed flower wither, resulting in a partial exposure of the fertilising organs. A defective development of the keel has also been observed, owing to which the stigma and anthers remained partially uncovered.² It also sometimes happens that the pollen does not reach full perfection. In this event there occurs a gradual lengthening of the pistil during the blooming period, until the stigmatic tip protrudes at the point of the keel. This remarkable appearance has also been observed in hybrids of *Phaseolus* and *Lathyrus*.

The risk of false impregnation by foreign pollen is, however, a very slight one with *Pisum*, and is quite incapable of disturbing the general result. Among more than 10,000 plants which were carefully examined there were only a very few cases where an indubitable false impregnation had occurred. Since in the greenhouse

¹ [It is somewhat surprising that no mention is made of thrips, which swarm in pea flowers. I had come to the conclusion that this is a real source of error and I see Laxton held the same opinion.]

² [This also happens in sweet peas.]

such a case was never remarked, it may well be supposed that *Bruchus pisi*, and possibly also the described abnormalities in the floral structure, were to blame.

[F₁] The Forms of the Hybrids¹

Experiments which in previous years were made with ornamental plants have already afforded evidence that the hybrids, as a rule, are not exactly intermediate between the parental species. With some of the more striking characters, those, for instance, which relate to the form and size of the leaves, the pubescence of the several parts, &c., the intermediate, indeed, is nearly always to be seen; in other cases, however, one of the two parental characters is so preponderant that it is difficult, or quite impossible, to detect the other in the hybrid.

This is precisely the case with the pea hybrids. In the case of each of the seven crosses the hybrid-character resembles² that of one of the parental forms so closely that the other either escapes observation completely or cannot be detected with certainty. This circumstance is of great importance in the determination and classification of the forms under which the offspring of the hybrids appear. Henceforth in this paper those characters which are transmitted entire, or almost unchanged in the hybridisation, and therefore in themselves constitute the characters of the hybrid, are termed the *dominant*, and those which become latent in the process *recessive*. The expression "recessive" has been chosen because the characters thereby designated withdraw or entirely disappear in the hybrids, but nevertheless reappear unchanged in their progeny, as will be demonstrated later on.

It was furthermore shown by the whole of the experiments that it is perfectly immaterial whether the dominant character belongs to the seed-bearer or to the pollen-parent; the form of the hybrid remains identical in both cases. This interesting fact was also emphasized by Gärtner, with the remark that even the most practised expert is not in a position to determine in a hybrid which of the two parental species was the seed or the pollen plant.³

Of the differentiating characters which were used in the experiments the following are dominant:

1. The round or roundish form of the seed with or without shallow depressions.
2. The yellow colouring of the seed albumen [cotyledons].
3. The grey, grey-brown, or leather-brown colour of the seed-coat, in association with violet-red blossoms and reddish spots in the leaf axils.
4. The simply inflated form of the pod.
5. The green colouring of the unripe pod in association with the same colour in the stems, the leaf-veins and the calyx.
6. The distribution of the flowers along the stem.
7. The greater length of stem.

¹ [Mendel throughout speaks of his cross-bred peas as "hybrids," a term which many restrict to the offspring of two distinct *species*. He, as he explains, held this to be only a question of degree.]

² [Note that Mendel, with true penetration, avoids speaking of the hybrid-character as "transmitted" by either parent, thus escaping the error pervading the older views of heredity.]

³ [Gärtner, p. 223.]

With regard to this last character it must be stated that the longer of the two parental stems is usually exceeded by the hybrid, a fact which is possibly only attributable to the greater luxuriance which appears in all parts of plants when stems of very different length are crossed. Thus, for instance, in repeated experiments, stems of 1 ft. and 6 ft. in length yielded without exception hybrids which varied in length between 6 ft. and $7\frac{1}{2}$ ft.

The hybrid seeds in the experiments with seed-coat are often more spotted and the spots sometimes coalesce into small bluish-violet patches. The spotting also frequently appears even when it is absent as a parental character.¹

The hybrid forms of the seed-shape and of the albumen [colour] are developed immediately after the artificial fertilisation by the mere influence of the foreign pollen. They can, therefore, be observed even in the first year of experiment, whilst all the other characters naturally only appear in the following year in such plants as have been raised from the crossed seed.

[F₂] The Generation [Bred] from the Hybrids

In this generation there reappear, together with the dominant characters, also the recessive ones with their peculiarities fully developed, and this occurs in the definitely expressed average proportion of three to one, so that among each four plants of this generation three display the dominant character and one the recessive. This relates without exception to all the characters which were investigated in the experiments. The angular wrinkled form of the seed, the green colour of the albumen, the white colour of the seed-coats and the flowers, the constrictions of the pods, the yellow colour of the unripe pod, of the stalk, of the calyx, and of the leaf venation, the umbel-like form of the inflorescence, and the dwarfed stem, all reappear in the numerical proportion given, without any essential alteration. *Transitional forms were not observed in any experiment.*

Since the hybrids resulting from reciprocal crosses are formed alike and present no appreciable difference in their subsequent development, consequently the results [of the reciprocal crosses] can be reckoned together in each experiment. The relative numbers which were obtained for each pair of differentiating characters are as follows:

Expt. 1. Form of seed.—From 253 hybrids 7,324 seeds were obtained in the second trial year. Among them were 5,474 round or roundish ones and 1,850 angular wrinkled ones. Therefrom the ratio 2.96 to 1 is deduced.

Expt. 2. Colour of albumen.—258 plants yielded 8,023 seeds, 6,022 yellow, and 2,001 green; their ratio, therefore, is as 3.01 to 1.

In these two experiments each pod yielded usually both kinds of seeds. In well-developed pods which contained on the average six to nine seeds, it often happened that all the seeds were round (Expt. 1) or all yellow (Expt. 2); on the other hand there were never observed more than five wrinkled or five green ones in one pod. It appears to make no difference whether the pods are developed early or later in the hybrid or whether they spring from the main axis or from a lateral one. In some few plants only a few seeds developed in the first formed pods, and these

¹ [This refers to the coats of the seeds borne by F₁ plants.]

possessed exclusively one of the two characters, but in the subsequently developed pods the normal proportions were maintained nevertheless.

As in separate pods, so did the distribution of the characters vary in separate plants. By way of illustration the first ten individuals from both series of experiments may serve.

EXPERIMENT 1.			EXPERIMENT 2.	
Form of Seed.			Colour of Albumen.	
Plants	Round	Angular	Yellow	Green
1	45	12	25	11
2	27	8	32	7
3	24	7	14	5
4	19	10	70	27
5	32	11	24	13
6	26	6	20	6
7	88	24	32	13
8	22	10	44	9
9	28	6	50	14
10	25	7	44	18

As extremes in the distribution of the two seed characters in one plant, there were observed in Expt. 1 an instance of 43 round and only 2 angular, and another of 14 round and 15 angular seeds. In Expt. 2 there was a case of 32 yellow and only 1 green seed, but also one of 20 yellow and 19 green.

These two experiments are important for the determination of the average ratios, because with a smaller number of experimental plants they show that very considerable fluctuations may occur. In counting the seeds, also, especially in Expt. 2, some care is requisite, since in some of the seeds of many plants the green colour of the albumen is less developed, and at first may be easily overlooked. The cause of this partial disappearance of the green colouring has no connection with the hybrid-character of the plants, as it likewise occurs in the parental variety. This peculiarity [bleaching] is also confined to the individual and is not inherited by the offspring. In luxuriant plants this appearance was frequently noted. Seeds which are damaged by insects during their development often vary in colour and form, but, with a little practice in sorting, errors are easily avoided. It is almost superfluous to mention that the pods must remain on the plants until they are thoroughly ripened and have become dried, since it is only then that the shape and colour of the seed are fully developed.

Expt. 3. Colour of the seed-coats.—Among 929 plants 705 bore violet-red flowers and grey-brown seed-coats; 224 had white flowers and white seed-coats, giving the proportion 3.15 to 1.

Expt. 4. Form of pods.—Of 1,181 plants 882 had them simply inflated, and in 299 they were constricted. Resulting ratio, 2.95 to 1.

Expt. 5. Colour of the unripe pods.—The number of trial plants was 580, of which 428 had green pods and 152 yellow ones. Consequently these stand in the ratio 2.82 to 1.

Expt. 6. Position of flowers.—Among 858 cases 651 had inflorescences axial and 207 terminal. Ratio, 3.14 to 1.

Expt. 7. Length of stem.—Out of 1,064 plants, in 787 cases the stem was long, and in 277 short. Hence a mutual ratio of 2.84 to 1. In this experiment the dwarfed plants were carefully lifted and transferred to a special bed. This precaution was necessary, as otherwise they would have perished through being overgrown by their tall relatives. Even in their quite young state they can be easily picked out by their compact growth and thick dark-green foliage.¹

If now the results of the whole of the experiments be brought together, there is found, as between the number of forms with the dominant and recessive characters, an average ratio of 2.98 to 1, or 3 to 1.

The dominant character can have here a *double signification*—viz. that of a parental character, or a hybrid-character.² In which of the two significations it appears in each separate case can only be determined by the following generation. As a parental character it must pass over unchanged to the whole of the offspring; as a hybrid-character, on the other hand, it must maintain the same behaviour as in the first generation [F_2].

[F_2] The Second Generation [Bred] from the Hybrids

Those forms which in the first generation [F_2] exhibit the recessive character do not further vary in the second generation [F_3] as regards this character; they remain constant in their offspring.

It is otherwise with those which possess the dominant character in the first generation [bred from the hybrids]. Of these *two-thirds* yield offspring which display the dominant and recessive characters in the proportion of 3 to 1, and thereby show exactly the same ratio as the hybrid forms, while only *one-third* remains with the dominant character constant.

The separate experiments yielded the following results:

Expt. 1. Among 565 plants which were raised from round seeds of the first generation, 193 yielded round seeds only, and remained therefore constant in this character; 372, however, gave both round and wrinkled seeds, in the proportion of 3 to 1. The number of the hybrids, therefore, as compared with the constants is 1.93 to 1.

Expt. 2. Of 519 plants which were raised from seeds whose albumen was of yellow colour in the first generation, 166 yielded exclusively yellow, while 353 yielded yellow and green seeds in the proportion of 3 to 1. There resulted, therefore, a division into hybrid and constant forms in the proportion of 2.13 to 1.

For each separate trial in the following experiments 100 plants were selected which displayed the dominant character in the first generation, and in order to ascertain the significance of this, ten seeds of each were cultivated.

Expt. 3. The offspring of 36 plants yielded exclusively grey-brown seed-coats, while of the offspring of 64 plants some had grey-brown and some had white.

Expt. 4. The offspring of 29 plants had only simply inflated pods; of the offspring of 71, on the other hand, some had inflated and some constricted.

¹ [This is true also of the dwarf or "Cupid" sweet peas.]

² [This paragraph presents the view of the hybrid-character as something incidental to the hybrid, and not "transmitted" to it—a true and fundamental conception here expressed probably for the first time.]

Expt. 5. The offspring of 40 plants had only green pods; of the offspring of 60 plants some had green, some yellow ones.

Expt. 6. The offspring of 33 plants had only axial flowers; of the offspring of 67, on the other hand, some had axial and some terminal flowers.

Expt. 7. The offspring of 28 plants inherited the long axis, and those of 72 plants some the long and some the short axis.

In each of these experiments a certain number of the plants came constant with the dominant character. For the determination of the proportion in which the separation of the forms with the constantly persistent character results, the two first experiments are of especial importance, since in these a larger number of plants can be compared. The ratios 1.93 to 1 and 2.13 to 1 gave together almost exactly the average ratio of 2 to 1. The sixth experiment gave a quite concordant result; in the others the ratio varies more or less, as was only to be expected in view of the smaller number of 100 trial plants. Experiment 5, which shows the greatest departure, was repeated, and then, in lieu of the ratio of 60 and 40, that of 65 and 35 resulted. *The average ratio of 2 to 1 appears, therefore, as fixed with certainty.* It is therefore demonstrated that, of those forms which possess the dominant character in the first generation, two-thirds have the hybrid-character, while one-third remains constant with the dominant character.

The ratio of 3 to 1, in accordance with which the distribution of the dominant and recessive characters results in the first generation, resolves itself therefore in all experiments into the ratio of 2:1:1 if the dominant character be differentiated according to its significance as a hybrid-character or as a parental one. Since the members of the first generation [F_2] spring directly from the seed of the hybrids [F_1], *it is now clear that the hybrids form seeds having one or other of the two differentiating characters,¹ and of these one-half develop again the hybrid form, while the other half yield plants which remain constant and receive the dominant or the recessive characters [respectively] in equal numbers.*

The Subsequent Generations [Bred] from the Hybrids

The proportions in which the descendants of the hybrids develop and split up in the first and second generations presumably hold good for all subsequent progeny. Experiments 1 and 2 have already been carried through six generations, 3 and 7 through five, and 4, 5, and 6 through four, these experiments being continued from the third generation with a small number of plants, and no departure from the rule has been perceptible. The offspring of the hybrids separated in each generation in the ratio of 2:1:1 into hybrids and constant forms.

If A be taken as denoting one of the two constant characters, for instance the dominant, a , the recessive, and Aa the hybrid from in which both are conjoined, the expression

$$A + 2Aa + a$$

shows the terms in the series for the progeny of the hybrids of two differentiating characters.

¹ This is the Principle of Segregation (cf. p. 31).

The observation made by Gärtner, Kölreuter, and others, that hybrids are inclined to revert to the parental forms, is also confirmed by the experiments described. It is seen that the number of the hybrids which arise from one fertilisation, as compared with the number of forms which become constant, and their progeny from generation to generation, is continually diminishing, but that nevertheless they could not entirely disappear. If an average equality of fertility in all plants in all generations be assumed, and if, furthermore, each hybrid forms seed of which one-half yields hybrids again, while the other half is constant to both characters in equal proportions, the ratio of numbers for the offspring in each generation is seen by the following summary, in which A and a denote again the two parental characters, and Aa the hybrid forms. For brevity's sake it may be assumed that each plant in each generation furnishes only 4 seeds.

Generation				RATIOS		
	A	Aa	a	$A : Aa : a$		
1	1	2	1	1 : 2 : 1		
2	6	4	6	3 : 2 : 3		
3	28	8	28	7 : 2 : 7		
4	120	16	120	15 : 2 : 15		
5	496	32	496	31 : 2 : 31		
n				$2^n - 1 : 2 : 2^n - 1$		

In the tenth generation, for instance, $2^n - 1 = 1,023$. There result, therefore, in each 2,048 plants which arise in this generation 1,023 with the constant dominant character, 1,023 with the recessive character, and only two hybrids.

The Offspring of Hybrids in Which Several Differentiating Characters Are Associated

In the experiments above described plants were used which differed only in one essential character.¹ The next task consisted in ascertaining whether the law of development discovered in these applied to each pair of differentiating characters when several diverse characters are united in the hybrid by crossing. As regards the form of the hybrids in these cases, the experiments showed throughout that this invariably more nearly approaches to that one of the two parental plants which possesses the greater number of dominant characters. If, for instance, the seed plant has a short stem, terminal white flowers, and simply inflated pods; the pollen plant, on the other hand, a long stem, violet-red flowers distributed along the stem, and constricted pods; the hybrid resembles the seed parent only in the form of the pod; in the other characters it agrees with the pollen parent. Should one of the two parental types possess only dominant characters, then the hybrid is scarcely or not at all distinguishable from it.

¹ [This statement of Mendel's in the light of present knowledge is open to some misconception. Though his work makes it evident that such varieties may exist, it is very unlikely that Mendel could have had seven pairs of varieties such that the members of each pair differed from each other in *only* one considerable character (*wesentliches Merkmal*). The point is probably of little theoretical or practical consequence, but a rather heavy stress is thrown on "*wesentlich*."]]

Two experiments were made with a considerable number of plants. In the first experiment the parental plants differed in the form of the seed and in the colour of the albumen; in the second in the form of the seed, in the colour of the albumen, and in the colour of the seed-coats. Experiments with seed characters give the result in the simplest and most certain way.

In order to facilitate study of the data in these experiments, the different characters of the seed plant will be indicated by *A, B, C*, those of the pollen plant by *a, b, c*, and the hybrid forms of the characters by *Aa, Bb*, and *Cc*.

Experiment 1

<i>AB</i> , seed parents;	<i>ab</i> , pollen parents;
<i>A</i> , form round;	<i>a</i> , form wrinkled;
<i>B</i> , albumen yellow.	<i>b</i> , albumen green.

The fertilised seeds appeared round and yellow like those of the seed parents. The plants raised therefrom yielded seeds of four sorts, which frequently presented themselves in one pod. In all, 556 seeds were yielded by 15 plants, and of these there were:

315 round and yellow,
101 wrinkled and yellow,
108 round and green,
32 wrinkled and green.

All were sown the following year. Eleven of the round yellow seeds did not yield plants, and three plants did not form seeds. Among the rest:

38 had round yellow seeds.....	<i>AB</i>
65 round yellow and green seeds.....	<i>ABb</i>
60 round yellow and wrinkled yellow seeds.....	<i>AaB</i>
138 round yellow and green, wrinkled yellow and green seeds.....	<i>AaBb</i>

From the wrinkled yellow seeds 96 resulting plants bore seed, of which:

28 had only wrinkled yellow seeds	<i>aB</i>
68 wrinkled yellow and green seeds	<i>aBb</i> .

From 108 round green seeds 102 resulting plants fruited, of which:

35 had only round green seeds	<i>Ab</i>
67 round and wrinkled green seeds	<i>Aab</i> .

The wrinkled green seeds yielded 30 plants which bore seeds all of like character; they remained constant *ab*.

The offspring of the hybrids appeared therefore under nine different forms, some of them in very unequal numbers. When these are collected and co-ordinated we find:

38	plants with the sign	<i>AB</i>
35	" " " "	<i>Ab</i>
28	" " " "	<i>aB</i>
30	" " " "	<i>ab</i>
65	" " " "	<i>ABb</i>
68	" " " "	<i>aBb</i>
60	" " " "	<i>AaB</i>
67	" " " "	<i>Aab</i>
138	" " " "	<i>AaBb</i>

The whole of the forms may be classed into three essentially different groups. The first includes those with the signs *AB*, *Ab*, *aB*, and *ab*: they possess only constant characters and do not vary again in the next generation. Each of these forms is represented on the average thirty-three times. The second group includes the signs *ABb*, *aBb*, *AaB*, *Aab*: these are constant in one character and hybrid in another, and vary in the next generation only as regards the hybrid-character. Each of these appears on an average sixty-five times. The form *AaBb* occurs 138 times: it is hybrid in both characters, and behaves exactly as do the hybrids from which it is derived.

If the numbers in which the forms belonging to these classes appear be compared, the ratios of 1, 2, 4 are unmistakably evident. The numbers 38, 65, 138 present very fair approximations to the ratio numbers of 33, 66, 132.

The developmental series consists, therefore, of nine classes, of which four appear therein always once and are constant in both characters; the forms *AB*, *ab*, resemble the parental forms, the two other present combinations between the conjoined characters *A*, *a*, *B*, *b*, which combinations are likewise possibly constant. Four classes appear always twice, and are constant in one character and hybrid in the other. One class appears four times, and is hybrid in both characters. Consequently the offspring of the hybrids, if two kinds of differentiating characters are combined therein, are represented by the expression

$$AB + Ab + aB + ab + 2ABb + 2aBb + 2AaB + 2Aab + 4AaBb.$$

This expression is indisputably a combination series in which the two expressions for the characters *A* and *a*, *B* and *b* are combined. We arrive at the full number of the classes of the series by the combination of the expressions:

$$\begin{aligned} A + 2Aa + a \\ B + 2Bb + b. \end{aligned}$$

Experiment 2

<i>ABC</i> , seed parents;	<i>abc</i> , pollen parents;
<i>A</i> , form round;	<i>a</i> , form wrinkled;
<i>B</i> , albumin yellow;	<i>b</i> , albumen green;
<i>C</i> , seed-coat grey-brown.	<i>c</i> , seed-coat white.

This experiment was made in precisely the same way as the previous one. Among all the experiments it demanded the most time and trouble. From 24 hybrids

687 seeds were obtained in all: these were all either spotted, grey-brown or grey-green, round or wrinkled.¹ From these in the following year 639 plants fruited, and, as further investigation showed, there were among them:

8 plants <i>ABC</i>	22 plants <i>ABCc</i>	45 plants <i>ABbCc</i>
14 " <i>ABc</i>	17 " <i>AbCc</i>	36 " <i>aBbCc</i>
9 " <i>AbC</i>	25 " <i>aBCc</i>	38 " <i>AaBCc</i>
11 " <i>Abc</i>	20 " <i>abCc</i>	40 " <i>AabCc</i>
8 " <i>aBC</i>	15 " <i>ABbC</i>	49 " <i>AaBbC</i>
10 " <i>aBc</i>	18 " <i>ABbc</i>	48 " <i>AaBbc</i>
10 " <i>abC</i>	19 " <i>aBbC</i>	
7 " <i>abc</i>	24 " <i>aBbc</i>	
	14 " <i>AaBC</i>	78 " <i>AaBbCc</i>
	18 " <i>AaBc</i>	
	20 " <i>AabC</i>	
	16 " <i>Aabc</i>	

The whole expression contains 27 terms. Of these 8 are constant in all characters, and each appears on the average 10 times; 12 are constant in two characters, and hybrid in the third; each appears on the average 19 times; 6 are constant in one character and hybrid in the other two; each appears on the average 43 times. One form appears 78 times and is hybrid in all of the characters. The ratios 10, 19, 43, 78 agree so closely with the ratios 10, 20, 40, 80, or 1, 2, 4, 8, that this last undoubtedly represents the true value.

The development of the hybrids when the original parents differ in three characters results therefore according to the following expression:

$$\begin{aligned}
 &ABC + ABc + AbC + Abc + aBC + aBc + abC + abc \\
 &+ 2 ABCc + 2 AbCc + 2 aBCc + 2 abCc + 2 ABbC \\
 &+ 2 ABbc + 2 aBbC + 2 aBbc + 2 AaBC + 2 AaBc \\
 &+ 2 AabC + 2 Aabc + 4 ABbCc + 4 aBbCc + 4 AaBCc \\
 &+ 4 AabCc + 4 AaBbC + 4 AaBbC + 8 AaBbCc.
 \end{aligned}$$

Here also is involved a combination series in which the expressions for the characters *A* and *a*, *B* and *b*, *C* and *c*, are united. The expressions

$$\begin{aligned}
 &A + 2Aa + a \\
 &B + 2Bb + b \\
 &C + 2Cc + c
 \end{aligned}$$

give all the classes of the series. The constant combinations which occur therein agree with all combinations which are possible between the characters *A*, *B*, *C*, *a*, *b*, *c*; two thereof, *ABC* and *abc*, resemble the two original parental stocks.

In addition, further experiments were made with a smaller number of experimental plants in which the remaining characters by twos and threes were united as hybrids: all yielded approximately the same results. There is therefore no doubt

¹ [Note that Mendel does not state the cotyledon-colour of the first crosses in this case; for as the coats were thick, it could not have been seen without opening or peeling the seeds.]

that for the whole of the characters involved in the experiments the principle applies that *the offspring of the hybrids in which several essentially different characters are combined exhibit the terms of a series of combinations, in which the developmental series for each pair of differentiating characters are united*. It is demonstrated at the same time that *the relation of each pair of different characters in hybrid union is independent of the other differences in the two original parental stocks*.¹

If n represents the number of the differentiating characters in the two original stocks, 3^n gives the number of terms of the combination series, 4^n the number of individuals which belong to the series, and 2^n the number of unions which remain constant. The series therefore contains, if the original stocks differ in four characters, $3^4 = 81$ classes, $4^4 = 256$ individuals, and $2^4 = 16$ constant forms; or which is the same, among each 256 offspring of the hybrids there are 81 different combinations, 16 of which are constant.

All constant combinations which in peas are possible by the combination of the said seven differentiating characters were actually obtained by repeated crossing. Their number is given by $2^7 = 128$. Thereby is simultaneously given the practical proof that *the constant characters which appear in the several varieties of a group of plants may be obtained in all the associations which are possible according to the [mathematical] laws of combination, by means of repeated artificial fertilisation*.

As regards the flowering time of the hybrids, the experiments are not yet concluded. It can, however, already be stated that the time stands almost exactly between those of the seed and pollen parents, and that the constitution of the hybrids with respect to this character probably follows the rule ascertained in the case of the other characters. The forms which are selected for experiments of this class must have a difference of at least twenty days from the middle flowering period of one to that of the other; furthermore, the seeds when sown must all be placed at the same depth in the earth, so that they may germinate simultaneously. Also, during the whole flowering period, the more important variations in temperature must be taken into account, and the partial hastening or delaying of the flowering which may result therefrom. It is clear that this experiment presents many difficulties to be overcome and necessitates great attention.

If we endeavour to collate in a brief form the results arrived at, we find that those differentiating characters, which admit of easy and certain recognition in the experimental plants, all behave exactly alike in their hybrid associations. The offspring of the hybrids of each pair of differentiating characters are, one-half, hybrid again, while the other half are constant in equal proportions having the characters of the seed and pollen parents respectively. If several differentiating characters are combined by cross-fertilisation in a hybrid, the resulting offspring form the terms of a combination series in which the combination series for each pair of differentiating characters are united.

The uniformity of behaviour shown by the whole of the characters submitted to experiment permits, and fully justifies, the acceptance of the principle that a similar relation exists in the other characters which appear less sharply defined in plants, and therefore could not be included in the separate experiments. An experi-

¹ This is the Principle of Independent Assortment (cf. p. 58).

ment with peduncles of different lengths gave on the whole a fairly satisfactory result, although the differentiation and serial arrangement of the forms could not be effected with that certainty which is indispensable for correct experiment.

The Reproductive Cells of the Hybrids

The results of the previously described experiments led to further experiments, the results of which appear fitted to afford some conclusions as regards the composition of the egg and pollen cells of hybrids. An important clue is afforded in *Pisum* by the circumstance that among the progeny of the hybrids constant forms appear, and that this occurs, too, in respect of all combinations of the associated characters. So far as experience goes, we find it in every case confirmed that constant progeny can only be formed when the egg cells and the fertilising pollen are of like character, so that both are provided with the material for creating quite similar individuals, as is the case with the normal fertilisation of pure species. We must therefore regard it as certain that exactly similar factors must be at work also in the production of the constant forms in the hybrid plants. Since the various constant forms are produced in *one* plant, or even in *one* flower of a plant, the conclusion appears logical that in the ovaries of the hybrids there are formed as many sorts of egg cells, and in the anthers as many sorts of pollen cells, as there are possible constant combination forms, and that these egg and pollen cells agree in their internal composition with those of the separate forms.

In point of fact it is possible to demonstrate theoretically that this hypothesis would fully suffice to account for the development of the hybrids in the separate generations, if we might at the same time assume that the various kinds of egg and pollen cells were formed in the hybrids on the average in equal numbers.¹

In order to bring these assumptions to an experimental proof, the following experiments were designed. Two forms which were constantly different in the form of the seed and the colour of the albumen were united by fertilisation.

If the differentiating characters are again indicated as *A*, *B*, *a*, *b*, we have:

<i>AB</i> , seed parent;	<i>ab</i> , pollen parent;
<i>A</i> , form round;	<i>a</i> , form wrinkled;
<i>B</i> , albumen yellow.	<i>b</i> , albumen green.

The artificially fertilised seeds were sown together with several seeds of both original stocks, and the most vigorous examples were chosen for the reciprocal crossing. There were fertilised:

1. The hybrids with the pollen of *AB*.
2. The hybrids " " " " *ab*.
3. *AB* " " " " the hybrids.
4. *ab* " " " " the hybrids.

For each of these four experiments the whole of the flowers on three plants were fertilised. If the above theory be correct, there must be developed on the hybrids

¹ [This and the preceding paragraph contain the essence of the Mendelian principles of heredity.]

egg and pollen cells of the forms AB , Ab , aB , ab , and there would be combined:

1. The egg cells AB , Ab , aB , ab with the pollen cells AB .
2. The egg cells AB , Ab , aB , ab with the pollen cells ab .
3. The egg cells AB with the pollen cells AB , Ab , aB , ab .
4. The egg cells ab with the pollen cells AB , Ab , aB , ab .

From each of these experiments there could then result only the following forms:

- | | |
|------------------------------------|------------------------------------|
| 1. AB , ABb , AaB , $AaBb$. | 3. AB , ABb , AaB , $AaBb$. |
| 2. $AaBb$, Aab , aBb , ab . | 4. $AaBb$, Aab , aBb , ab . |

If, furthermore, the several forms of the egg and pollen cells of the hybrids were produced on an average in equal numbers, then in each experiment the said four combinations should stand in the same ratio to each other. A perfect agreement in the numerical relations was, however, not to be expected, since in each fertilisation, even in normal cases, some egg cells remain undeveloped or subsequently die, and many even of the well-formed seeds fail to germinate when sown. The above assumption is also limited in so far that, while it demands the formation of an equal number of the various sorts of egg and pollen cells, it does not require that this should apply to each separate hybrid with mathematical exactness.

The first and second experiments had primarily the object of proving the composition of the hybrid egg cells, while the third and fourth experiments were to decide that of the pollen cells.¹ As is shown by the above demonstration the first and third experiments and the second and fourth experiments should produce precisely the same combinations, and even in the second year the result should be partially visible in the form and colour of the artificially fertilised seed. In the first and third experiments the dominant characters of form and colour, A and B , appear in each union, and are also partly constant and partly in hybrid union with the recessive characters a and b , for which reason they must impress their peculiarity upon the whole of the seeds. All seeds should therefore appear round and yellow, if the theory be justified. In the second and fourth experiments, on the other hand, one union is hybrid in form and in colour, and consequently the seeds are round and yellow; another is hybrid in form, but constant in the recessive character of colour, whence the seeds are round and green; the third is constant in the recessive character of form but hybrid in colour, consequently the seeds are wrinkled and yellow; the fourth is constant in both recessive characters, so that the seeds are wrinkled and green. In both these experiments there were consequently four sorts of seed to be expected—viz. round and yellow, round and green, wrinkled and yellow, wrinkled and green.

The crop fulfilled these expectations perfectly. There were obtained in the

1st Experiment, 98 exclusively round yellow seeds;
 3rd " 94 " " " " "

¹ [To prove, namely, that both were similarly differentiated, and not one or other only.]

In the 2d Experiment, 31 round and yellow, 26 round and green, 27 wrinkled and yellow, 26 wrinkled and green seeds.

In the 4th Experiment, 24 round and yellow, 25 round and green, 22 wrinkled and yellow, 26 wrinkled and green seeds.

There could scarcely be now any doubt of the success of the experiment; the next generation must afford the final proof. From the seed sown there resulted for the first experiment 90 plants, and for the third 87 plants which fruited: these yielded for the

1st Exp. 3rd Exp.

20	25	round yellow seeds.....	<i>AB</i>
23	19	round yellow and green seeds.....	<i>ABb</i>
25	22	round and wrinkled yellow seeds.....	<i>AaB</i>
22	21	round and wrinkled green and yellow seeds.....	<i>AaBb</i>

In the second and fourth experiments the round and yellow seeds yielded plants with round and wrinkled yellow and green seeds, *AaBb*.

From the round green seeds, plants resulted with round and wrinkled green seeds, *Aab*.

The wrinkled yellow seeds gave plants with wrinkled yellow and green seeds, *aBb*.

From the wrinkled green seeds plants were raised which yielded again only wrinkled and green seeds, *ab*.

Although in these two experiments likewise some seeds did not germinate, the figures arrived at already in the previous year were not affected thereby, since each kind of seed gave plants which, as regards their seed, were like each other and different from the others. There resulted therefore from the

2nd Exp. 4th Exp.

31	24	plants of the form <i>AaBb</i>
26	25	" " " " <i>Aab</i>
27	22	" " " " <i>aBb</i>
26	27	" " " " <i>ab</i>

In all the experiments, therefore, there appeared all the forms which the proposed theory demands, and they came in nearly equal numbers.

In a further experiment the characters of flower-colour and length of stem were experimented upon, and selection was so made that in the third year of the experiment each character ought to appear in half of all the plants if the above theory were correct. *A*, *B*, *a*, *b* serve again as indicating the various characters.

<i>A</i> , violet-red flowers.	<i>a</i> , white flowers.
<i>B</i> , axis long.	<i>b</i> , axis short.

The form *Ab* was fertilised with *ab*, which produced the hybrid *Aab*. Furthermore, *aB* was also fertilised with *ab*, whence the hybrid *aBb*. In the second year, for further fertilisation, the hybrid *Aab* was used as seed parent, and hybrid *aBb* as pollen parent.

Seed parent, Aab .	Pollen parent, aBb .
Possible egg cells, Ab, ab .	Pollen cells, aB, ab .

From the fertilisation between the possible egg and pollen cells four combinations should result, viz.,

$$AaBb + aBb + Aab + ab.$$

From this it is perceived that, according to the above theory, in the third year of the experiment out of all the plants

Half should have violet-red flowers (Aa),	Classes 1, 3
“ “ “ white flowers (a)	“ 2, 4
“ “ “ a long axis (Bb)	“ 1, 2
“ “ “ a short axis (b)	“ 3, 4

From 45 fertilisations of the second year 187 seeds resulted, of which only 166 reached the flowering stage in the third year. Among these the separate classes appeared in the numbers following:

Class	Colour of flower	Stem	
1	violet-red	long	47 times
2	white	long	40 “
3	violet-red	short	38 “
4	white	short	41 “

There subsequently appeared

The violet-red flower-colour (Aa) in 85 plants.
“ white “ “ (a) in 81 “
“ long stem (Bb) in 87 “
“ short “ (b) in 79 “

The theory adduced is therefore satisfactorily confirmed in this experiment also.

For the characters of form of pod, colour of pod, and position of flowers, experiments were also made on a small scale, and results obtained in perfect agreement. All combinations which were possible through the union of the differentiating characters duly appeared, and in nearly equal numbers.

Experimentally, therefore, the theory is confirmed that *the pea hybrids form egg and pollen cells which, in their constitution, represent in equal numbers all constant forms which result from the combination of the characters united in fertilisation.*

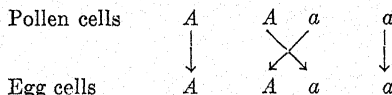
The difference of the forms among the progeny of the hybrids, as well as the respective ratios of the numbers in which they are observed, find a sufficient explanation in the principle above deduced. The simplest case is afforded by the developmental series of each pair of differentiating characters. This series is represented by the expression $A + 2Aa + a$, in which A and a signify the forms with constant differentiating characters, and Aa the hybrid form of both. It includes in three different classes four individuals. In the formation of these, pollen and egg cells of the form A and a take part on the average equally in the fertilisation; hence each form [occurs] twice, since four individuals are formed. There participate

consequently in the fertilisation

The pollen cells $A + A + a + a$

The egg cells $A + A + a + a$

It remains, therefore, purely a matter of chance which of the two sorts of pollen will become united with each separate egg cell. According, however, to the law of probability, it will always happen, on the average of many cases, that each pollen form, A and a , will unite equally often with each egg cell form, A and a , consequently one of the two pollen cells A in the fertilisation will meet with the egg cell A and the other with an egg cell a , and so likewise one pollen cell a will unite with an egg cell A , and the other with egg cell a .



The result of the fertilisation may be made clear by putting the signs for the conjoined egg and pollen cells in the form of fractions, those for the pollen cells above and those for the egg cells below the line. We have then

$$\frac{A}{A} + \frac{A}{a} + \frac{a}{A} + \frac{a}{a}$$

In the first and fourth term the egg and pollen cells are of like kind, consequently the product of their union must be constant, viz. A and a ; in the second and third, on the other hand, there again results a union of the two differentiating characters of the stocks, consequently the forms resulting from these fertilisations are identical with those of the hybrid from which they sprang. *There occurs accordingly a repeated hybridisation.* This explains the striking fact that the hybrids are able to produce, besides the two parental forms, offspring which are like themselves; $\frac{A}{a}$ and $\frac{a}{A}$ both give the same union Aa , since, as already remarked above, it makes no difference in the result of fertilisation to which of the two characters the pollen or egg cells belong. We may write then

$$\frac{A}{A} + \frac{A}{a} + \frac{a}{A} + \frac{a}{a} = A + 2Aa + a.$$

This represents the average result of the self-fertilisation of the hybrids when two differentiating characters are united in them. In individual flowers and in individual plants, however, the ratios in which the forms of the series are produced may suffer not inconsiderable fluctuations.¹ Apart from the fact that the numbers in which both sorts of egg cells occur in the seed vessels can only be regarded as equal on the average, it remains purely a matter of chance which of the two sorts of pollen may fertilise each separate egg cell. For this reason the separate values

¹ [Whether segregation by such units is more than purely fortuitous may perhaps be determined by seriation.]

must necessarily be subject to fluctuations, and there are even extreme cases possible, as were described earlier in connection with the experiments on the form of the seed and the colour of the albumen. The true ratios of the numbers can only be ascertained by an average deduced from the sum of as many single values as possible; the greater the number, the more are merely chance effects eliminated.

The developmental series for hybrids in which two kinds of differentiating characters are united contains, among sixteen individuals, nine different forms, viz.,

$$AB + Ab + aB + ab + 2ABb + 2aBb + 2AaB + 2Aab + 4AaBb.$$

Between the differentiating characters of the original stocks, *Aa* and *Bb*, four constant combinations are possible, and consequently the hybrids produce the corresponding four forms of egg and pollen cells *AB*, *Ab*, *aB*, *ab*, and each of these will on the average figure four times in the fertilisation, since sixteen individuals are included in the series. Therefore the participators in the fertilisation are

$$\begin{array}{l} \text{Pollen cells } AB + AB + AB + AB + Ab + Ab + Ab + Ab \\ \quad + aB + aB + aB + aB + ab + ab + ab + ab. \end{array}$$

$$\begin{array}{l} \text{Egg cells } AB + AB + AB + AB + Ab + Ab + Ab + Ab \\ \quad + aB + aB + aB + aB + ab + ab + ab + ab. \end{array}$$

In the process of fertilisation each pollen form unites on an average equally often with each egg cell form, so that each of the four pollen cells *AB* unites once with one of the forms of egg cell *AB*, *Ab*, *aB*, *ab*. In precisely the same way the rest of the pollen cells of the forms, *Ab*, *aB*, *ab* unite with all the other egg cells. We obtain therefore

$$\begin{array}{l} \frac{AB}{AB} + \frac{AB}{Ab} + \frac{AB}{aB} + \frac{AB}{ab} + \frac{Ab}{AB} + \frac{Ab}{Ab} + \frac{Ab}{aB} + \frac{Ab}{ab} \\ \quad + \frac{aB}{AB} + \frac{aB}{Ab} + \frac{aB}{aB} + \frac{aB}{ab} + \frac{ab}{AB} + \frac{ab}{Ab} + \frac{ab}{aB} + \frac{ab}{ab} \end{array}$$

or

$$\begin{array}{l} AB + ABb + AaB + AaBb + ABb + Ab + AaBb + Aab + AaB \\ \quad + AaBb + aB + aBb + AaBb + Aab + aBb + ab = AB \\ \quad + Ab + aB + ab + 2ABb + 2aBb + 2AaB + 2Aab + 4AaBb.^1 \end{array}$$

In precisely similar fashion is the developmental series of hybrids exhibited when three kinds of differentiating characters are conjoined in them. The hybrids form eight various kinds of egg and pollen cells—*ABC*, *ABc*, *AbC*, *Abc*, *aBC*, *aBc*, *abC*, *abc*—and each pollen form unites itself again on the average once with each form of egg cell.

The law of combination of different characters, which governs the development of the hybrids finds therefore its foundation and explanation in the principle enunciated, that the hybrids produce egg cells and pollen cells which in equal num-

¹ [In the original the sign of equality (=) is here represented by +, evidently a misprint.]

bers represent all constant forms which result from the combination of the characters brought together in fertilisation.

Experiments with Hybrids of Other Species of Plants

It must be the object of further experiments to ascertain whether the law of development discovered for *Pisum* applies also to the hybrids of other plants. To this end several experiments were recently commenced. Two minor experiments with species of *Phaseolus* have been completed, and may be here mentioned.

An experiment with *Phaseolus vulgaris* and *Phaseolus nanus* gave results in perfect agreement. *Ph. nanus* had, together with the dwarf axis, simply inflated, green pods. *Ph. vulgaris* had, on the other hand, an axis 10 feet to 12 feet high, and yellow-coloured pods, constricted when ripe. The ratios of the numbers in which the different forms appeared in the separate generations were the same as with *Pisum*. Also the development of the constant combinations resulted according to the law of simple combination of characters, exactly as in the case of *Pisum*. There were obtained

Constant combinations	Axis	Colour of the unripe pods	Form of the ripe pods
1	long	green	inflated
2	"	"	constricted
3	"	yellow	inflated
4	"	"	constricted
5	short	green	inflated
6	"	"	constricted
7	"	yellow	inflated
8	"	"	constricted

The green colour of the pod, the inflated forms, and the long axis were, as in *Pisum*, dominant characters.

Another experiment with two very different species of *Phaseolus* had only a partial result. *Phaseolus nanus*, L., served as seed parent, a perfectly constant species, with white flowers in short racemes and small white seeds in straight, inflated, smooth pods; as pollen parent was used *Ph. multiflorus*, W., with tall winding stem, purple-red flowers in very long racemes, rough, sickle-shaped crooked pods, and large seeds which bore black flecks and splashes on a peach-blood-red ground.

The hybrids had the greatest similarity to the pollen parent, but the flowers appeared less intensely coloured. Their fertility was very limited; from seventeen plants, which together developed many hundreds of flowers, only forty-nine seeds in all were obtained. These were of medium size, and were flecked and splashed similarly to those of *Ph. multiflorus*, while the ground colour was not materially different. The next year forty-four plants were raised from these seeds, of which only thirty-one reached the flowering stage. The characters of *Ph. nanus*, which had been altogether latent in the hybrids, reappeared in various combinations; their ratio, however, with relation to the dominant plants was necessarily very fluctuating owing to the small number of trial plants. With certain characters,

as in those of the axis and the form of pod it was, however, as in the case of *Pisum*, almost exactly 1:3.

Insignificant as the results of this experiment may be as regards the determination of the relative numbers in which the various forms appeared, it presents, on the other hand, the phenomenon of a remarkable change of colour in the flowers and seed of the hybrids. In *Pisum* it is known that the characters of the flower- and seed-colour present themselves unchanged in the first and further generations, and that the offspring of the hybrids display exclusively the one or the other of the characters of the original stocks. It is otherwise in the experiment we are considering. The white flowers and the seed-colour of *Ph. nanus* appeared, it is true, at once in the first generation [from the hybrids] in one fairly fertile example, but the remaining thirty plants developed flower-colours which were of various grades of purple-red to pale violet. The colouring of the seed-coat was no less varied than that of the flowers. No plant could rank as fully fertile; many produced no fruit at all; others only yielded fruits from the flowers last produced, which did not ripen. From fifteen plants only were well-developed seeds obtained. The greatest disposition to infertility was seen in the forms with preponderantly red flowers, since out of sixteen of these only four yielded ripe seeds. Three of these had a similar seed pattern to *Ph. multiflorus*, but with a more or less pale ground colour; the fourth plant yielded only one seed of plain brown tint. The forms with preponderantly violet-coloured flowers had dark brown, black-brown, and quite black seeds.

The experiment was continued through two more generations under similar unfavorable circumstances, since even among the offspring of fairly fertile plants there came again some which were less fertile or even quite sterile. Other flower- and seed-colours than those cited did not subsequently present themselves. The forms which in the first generation [bred from the hybrids] contained one or more of the recessive characters remained, as regards these, constant without exception. Also of those plants which possessed violet flowers and brown or black seed, some did not vary again in these respects in the next generation; the majority, however, yielded, together with offspring exactly like themselves, some which displayed white flowers and white seed-coats. The red flowering plants remained so slightly fertile that nothing can be said with certainty as regards their further development.

Despite the many disturbing factors with which the observations had to contend, it is nevertheless seen by this experiment that the development of the hybrids, with regard to those characters which concern the form of the plants, follows the same laws as in *Pisum*. With regard to the colour characters, it certainly appears difficult to perceive a substantial agreement. Apart from the fact that from the union of a white and a purple-red colouring a whole series of colours results [in F_2], from purple to pale violet and white, the circumstance is a striking one that among thirty-one flowering plants only one received the recessive character of the white colour, while in *Pisum* this occurs on the average in every fourth plant.

Even these enigmatical results, however, might probably be explained by the law governing *Pisum* if we might assume that the colour of the flowers and seeds of *Ph. multiflorus* is a combination of two or more entirely independent colours, which individually act like any other constant character in the plant. If the

flower-colour A were a combination of the individual characters $A_1 + A_2 + \dots$ which produce the total impression of a purple coloration, then by fertilisation with the differentiating character, white colour, a , there would be produced the hybrid unions $A_1a + A_2a + \dots$ and so would it be with the corresponding colouring of the seed-coats.¹ According to the above assumption, each of these hybrid colour unions would be independent, and would consequently develop quite independently from the others. It is then easily seen that from the combination of the separate developmental series a complete colour-series must result. If, for instance, $A = A_1 + A_2$, then the hybrids A_1a and A_2a form the developmental series—

$$A_1 + 2A_1a + a, \quad A_2 + 2A_2a + a.$$

The members of this series can enter into nine different combinations, and each of these denotes another colour—

1 A_1A_2	2 A_1A_2a	1 A_2a
2 A_1A_2a	4 A_1A_2aa	2 A_2aa
1 A_1a	2 A_1aa	1 aa .

The figures prescribed for the separate combinations also indicate how many plants with the corresponding colouring belong to the series. Since the total is sixteen, the whole of the colours are on the average distributed over each sixteen plants, but, as the series itself indicates, in unequal proportions.

Should the colour development really happen in this way, we could offer an explanation of the case above described, viz. that the white flowers and seed-coat colour only appeared once among thirty-one plants of the first generation. This colouring appears only once in the series, and could therefore also only be developed once in the average in each sixteen, and with three colour characters only once even in sixty-four plants.

It must, nevertheless, not be forgotten that the explanation here attempted is based on a mere hypothesis, only supported by the very imperfect result of the experiment just described. It would, however, be well worth while to follow up the development of colour in hybrids by similar experiments, since it is probable that in this way we might learn the significance of the extraordinary variety in the colouring of our ornamental flowers.

So far, little at present is known with certainty beyond the fact that the colour of the flowers in most ornamental plants is an extremely variable character. The opinion has often been expressed that the stability of the species is greatly disturbed or entirely upset by cultivation, and consequently there is an inclination to regard the development of cultivated forms as a matter of chance devoid of rules; the colouring of ornamental plants is indeed usually cited as an example of great instability. It is, however, not clear why the simple transference into garden soil should result in such a thorough and persistent revolution in the plant organism.

¹ [As it fails to take account of factors introduced by the albino this representation is imperfect. It is however interesting to know that Mendel realized the fact of the existence of compound characters, and that the rarity of the white recessives was a consequence of this resolution.]

No one will seriously maintain that in the open country the development of plants is ruled by other laws than in the garden bed. Here, as there, changes of type must take place if the conditions of life be altered, and the species possesses the capacity of fitting itself to its new environment. It is willingly granted that by cultivation the origination of new varieties is favoured, and that by man's labour many varieties are acquired which, under natural conditions, would be lost; but nothing justifies the assumption that the tendency to the formation of varieties is so extraordinarily increased that the species speedily lose all stability, and their offspring diverge into an endless series of extremely variable forms. Were the change in the conditions the sole cause of variability we might expect that those cultivated plants which are grown for centuries under almost identical conditions would again attain constancy. That, as is well known, is not the case, since it is precisely under such circumstances that not only the most varied but also the most variable forms are found. It is only the *Leguminosae*, like *Pisum*, *Phaseolus*,¹ *Lens*, whose organs of fertilisation are protected by the keel, which constitute a noteworthy exception. Even here there have arisen numerous varieties during a cultural period of more than 1,000 years under most various conditions; these maintain, however, under unchanging environments a stability as great as that of species growing wild.

It is more than probable that as regards the variability of cultivated plants there exists a factor which so far has received little attention. Various experiments force us to the conclusion that our cultivated plants, with few exceptions, are members of various hybrid series, whose further development in conformity with law is varied and interrupted by frequent crossings *inter se*. The circumstance must not be overlooked that cultivated plants are mostly grown in great numbers and close together, affording the most favourable conditions for reciprocal fertilisation between the varieties present and the species itself. The probability of this is supported by the fact that among the great array of variable forms solitary examples are always found, which in one character or another remain constant, if only foreign influence be carefully excluded. These forms behave precisely as do those which are known to be members of the compound hybrid series. Also with the most susceptible of all characters, that of colour, it cannot escape the careful observer that in the separate forms the inclination to vary is displayed in very different degrees. Among plants which arise from one spontaneous fertilisation there are often some whose offspring vary widely in the constitution and arrangement of the colours, while that of others shows little deviation, and among a greater number solitary examples occur which transmit the colour of the flowers unchanged to their offspring. The cultivated species of *Dianthus* afford an instructive example of this. A white-flowered example of *Dianthus caryophyllus*, which itself was derived from a white-flowered variety, was shut up during its blooming period in a greenhouse; the numerous seeds obtained therefrom yielded plants entirely white-flowered like itself. A similar result was obtained from a sub-species, with red flowers somewhat flushed with violet, and one with flowers white, striped with red. Many others, on the other hand, which were similarly protected, yielded progeny which were more or less variously coloured and marked.

¹ [*Phaseolus* nevertheless is insect-fertilised.]

Whoever studies the colouration which results, in ornamental plants, from similar fertilisation, can hardly escape the conviction that here also the development follows a definite law, which possibly finds its expression in the combination of several independent colour characters.

Concluding Remarks

It can hardly fail to be of interest to compare the observations made regarding *Pisum* with the results arrived at by the two authorities in this branch of knowledge, Kölreuter and Gärtner, in their investigations. According to the opinion of both, the hybrids in outward appearance present either a form intermediate between the original species, or they closely resemble either the one or the other type, and sometimes can hardly be discriminated from it. From their seeds usually arise, if the fertilisation was effected by their own pollen, various forms which differ from the normal type. As a rule, the majority of individuals obtained by one fertilisation maintain the hybrid form, while some few others come more like the seed parent, and one or other individual approaches the pollen parent. This, however, is not the case with all hybrids without exception. Sometimes the offspring have more nearly approached, some the one and some the other of the two original stocks, or they all incline more to one or the other side; while in other cases they remain perfectly like the hybrid and continue constant in their offspring. The hybrids of varieties behave like hybrids of species, but they possess greater variability of form and a more pronounced tendency to revert to the original types.

With regard to the form of the hybrids and their development, as a rule an agreement with the observations made in *Pisum* is unmistakable. It is otherwise with the exceptional cases cited. Gärtner confesses even that the exact determination whether a form bears a greater resemblance to one or to the other of the two original species often involved great difficulty, so much depending upon the subjective point of view of the observer. Another circumstance could, however, contribute to render the results fluctuating and uncertain, despite the most careful observation and differentiation. For the experiments, plants were mostly used which rank as good species and are differentiated by a large number of characters. In addition to the sharply defined characters, where it is a question of greater or less similarity, those characters must also be taken into account which are often difficult to define in words, but yet suffice, as every plant specialist knows, to give the forms a peculiar appearance. If it be accepted that the development of hybrids follows the law which is valid for *Pisum*, the series in each separate experiment must contain very many forms, since the number of the terms, as is known, increases, with the number of the differentiating characters, as the powers of three. With a relatively small number of experimental plants the result therefore could only be approximately right, and in single cases might fluctuate considerably. If, for instance, the two original stocks differ in seven characters, and 100 or 200 plants were raised from the seeds of their hybrids to determine the grade of relationship of the offspring, we can easily see how uncertain the decision must become, since for seven differentiating characters the combination series contain 16,384 individuals under 2,187 various forms; now one and then another relationship could assert its predominance, just according as chance presented this or that form to the observer in a majority of cases.

If, furthermore, there appear among the differentiating characters at the same time *dominant* characters, which are transmitted entire or nearly unchanged to the hybrids, then in the terms of the developmental series that one of the two original parents which possesses the majority of dominant characters must always be predominant. In the experiment described relative to *Pisum*, in which three kinds of differentiating characters were concerned, all the dominant characters belonged to the seed parent. Although the terms of the series in their internal composition approach both original parents equally, yet in this experiment the type of the seed parent obtained so great a preponderance that out of each sixty-four plants of the first generation fifty-four exactly resembled it, or only differed in one character. It is seen how rash it must be under such circumstances to draw from the external resemblances of hybrids conclusions as to their internal nature.

Gärtner mentions that in those cases where the development was regular, among the offspring of the hybrids, the two original species were not reproduced, but only a few individuals which approached them. With very extended developmental series it could not in fact be otherwise. For seven differentiating characters, for instance, among more than 16,000 individuals—offspring of the hybrids—each of the two original species would occur only once. It is therefore hardly possible that these should appear at all among a small number of experimental plants; with some probability, however, we might reckon upon the appearance in the series of a few forms which approach them.

We meet with an *essential difference* in those hybrids which remain constant in their progeny and propagate themselves as truly as the pure species. According to Gärtner, to this class belong the *remarkably fertile hybrids*, *Aquilegia atropurpurea canadensis*, *Lavatera pseudolbia thuringiaca*, *Geum urbano-rivale*, and some *Dianthus* hybrids; and, according to Wichura, the hybrids of the Willow family. For the history of the evolution of plants this circumstance is of special importance, since constant hybrids acquire the status of new species. The correctness of the facts is guaranteed by eminent observers, and cannot be doubted. Gärtner had an opportunity of following up *Dianthus Armeria deltoides* to the tenth generation, since it regularly propagated itself in the garden.

With *Pisum* it was shown by experiment that the hybrids form egg and pollen cells of *different* kinds, and that herein lies the reason of the variability of their offspring. In other hybrids, likewise, whose offspring behave similarly we may assume a like cause; for those, on the other hand, which remain constant, the assumption appears justifiable that their reproductive cells are all alike and agree with the foundation-cell [fertilised ovum] of the hybrid. In the opinion of renowned physiologists, for the purpose of propagation one pollen cell and one egg cell unite in Phanerogams¹ into a single cell, which is capable by assimilation and formation of new cells to become an independent organism. This development follows a constant law, which is founded on the material composition and arrangement of the elements which meet in the cell in a vivifying union. If the repro-

¹ In *Pisum* it is placed beyond doubt that for the formation of the new embryo a perfect union of the elements of both reproductive cells must take place. How could we otherwise explain that among the offspring of the hybrids both original types reappear in equal numbers and with all their peculiarities? If the influence of the egg cell upon the pollen cell were only external, if it fulfilled the rôle of a nurse

ductive cells be of the same kind and agree with the foundation cell [fertilised ovum] of the mother plant, then the development of the new individual will follow the same law which rules the mother plant. If it chance that an egg cell unites with a *dissimilar* pollen cell, we must then assume that between those elements of both cells, which determine opposite characters, some sort of compromise is effected. The resulting compound cell becomes the foundation of the hybrid organism, the development of which necessarily follows a different scheme from that obtaining in each of the two original species. If the compromise be taken to be a complete one, in the sense, namely, that the hybrid embryo is formed from two similar cells, in which the differences are *entirely and permanently accommodated* together, the further result follows that the hybrids, like any other stable plant species, reproduce themselves truly in their offspring. The reproductive cells which are formed in their seed vessels and anthers are of one kind, and agree with the fundamental compound cell [fertilised ovum].

With regard to those hybrids whose progeny is *variable* we may perhaps assume that between the differentiating elements of the egg and pollen cells there also occurs a compromise, in so far that the formation of a cell as foundation of the hybrid becomes possible; but, nevertheless, the arrangement between the conflicting elements is only temporary and does not endure throughout the life of the hybrid plant. Since, in the habit of the plant, no changes are perceptible during the whole period of vegetation, we must further assume that it is only possible for the differentiating elements to liberate themselves from the enforced union when the fertilising cells are developed. In the formation of these cells all existing elements participate, in an entirely free and equal arrangement, by which it is only the differentiating ones which mutually separate themselves. In this way the production would be rendered possible of as many sorts of egg and pollen cells as there are combinations possible of the formative elements.

The attribution attempted here of the essential difference in the development of hybrids to a *permanent or temporary union* of the differing cell elements can, of course, only claim the value of an hypothesis for which the lack of definite data offers a wide scope. Some justification of the opinion expressed lies in the evidence afforded by *Pisum* that the behavior of each pair of differentiating characters in hybrid union is independent of the other differences between the two original plants, and, further, that the hybrid produces just so many kinds of egg and pollen cells as there are possible constant combination forms. The differentiating characters of two plants can finally, however, only depend upon differences in the composition and grouping of the elements which exist in the foundation-cells [fertilised ova] of the same in vital interaction.¹

only, then the result of each artificial fertilisation could be no other than that the developed hybrid should exactly resemble the pollen parent, or at any rate do so very closely. This the experiments so far have in no wise confirmed. An evident proof of the complete union of the contents of both cells is afforded by the experience gained on all sides that it is immaterial, as regards the form of the hybrid, which of the original species is the seed parent or which the pollen parent.

¹ "Welche in den Grundzellen derselben in lebendiger Wechselwirkung stehen."

Even the validity of the law formulated for *Pisum* requires still to be confirmed, and a repetition of the more important experiments is consequently much to be desired, that, for instance, relating to the composition of the hybrid fertilising cells. A differential [element] may easily escape the single observer,¹ which although at the outset may appear to be unimportant, may yet accumulate to such an extent that it must not be ignored in the total result. Whether the variable hybrids of other plant species observe an entire agreement must also be first decided experimentally. In the meantime we may assume that in material points an essential difference can scarcely occur, since the unity in the developmental plan of organic life is beyond question.

In conclusion, the experiments carried out by Kölreuter, Gärtner, and others with respect to the transformation of one species into another by artificial fertilisation merit special mention. Particular importance has been attached to those experiments and Gärtner reckons them among "the most difficult of all in hybridisation."

If a species *A* is to be transformed into a species *B*, both must be united by fertilisation and the resulting hybrids then be fertilised with the pollen of *B*; then, out of the various offspring resulting, that form would be selected which stood in nearest relation to *B* and once more be fertilised with *B* pollen, and so continuously until finally a form is arrived at which is like *B* and constant in its progeny. By this process the species *A* would change into the species *B*. Gärtner alone has effected thirty such experiments with plants of genera *Aquilegia*, *Dianthus*, *Geum*, *Lavatera*, *Lychnis*, *Malva*, *Nicotiana*, and *Oenothera*. The period of transformation was not alike for all species. While with some a triple fertilisation sufficed, with others this had to be repeated five or six times, and even in the same species fluctuations were observed in various experiments. Gärtner ascribes this difference to the circumstance that "the specific [*typische*] power by which a species, during reproduction, effects the change and transformation of the maternal type varies considerably in different plants, and that, consequently, the periods within which the one species is changed into the other must also vary, as also the number of generations, so that the transformation in some species is perfected in more, and in others in fewer generations." Further, the same observer remarks "that in these transformation experiments a good deal depends upon which type and which individual be chosen for further transformation."

If it may be assumed that in these experiments the constitution of the forms resulted in a similar way to that of *Pisum*, the entire process of transformation would find a fairly simple explanation. The hybrid forms as many kinds of egg cells as there are constant combinations possible of the characters conjoined therein, and one of these is always of the same kind as that of the fertilising pollen cells. Consequently there always exists the possibility with all such experiments that even from the second fertilisation there may result a constant form identical with that of the pollen parent. Whether this really be obtained depends in each separate case upon the number of the experimental plants, as well as upon the number of differentiating characters which are united by the fertilisation. Let us, for instance, assume that the plants selected for experiment differed in three characters,

¹ "Dem einzelnen Beobachter kann leicht ein Differenziale entgehen."

and the species ABC is to be transformed into the other species abc by repeated fertilisation with the pollen of the latter; the hybrids resulting from the first cross form eight different kinds of egg cells, viz.,

$$ABC, ABc, AbC, aBC, Abc, aBc, abC, abc.$$

These in the second year of experiment are united again with the pollen cells abc , and we obtain the series

$$AaBbCc + AaBbc + AabCc + aBbCc + Aabc + aBbc + abCc + abc.$$

Since the form abc occurs once in the series of eight terms, it is consequently little likely that it would be missing among the experimental plants, even were these raised in a smaller number, and the transformation would be perfected already by a second fertilisation. If by chance it did not appear, then the fertilisation must be repeated with one of those forms nearest akin, $Aabc$, $aBbc$, $abCc$. It is perceived that such an experiment must extend the farther *the smaller the number of experimental plants and the larger the number of differentiating characters* in the two original species; and that, furthermore, in the same species there can easily occur a delay of one or even of two generations such as Gärtner observed. The transformation of widely divergent species could generally only be completed in five or six years of experiment, since the number of different egg cells which are formed in the hybrid increases, as the powers of two, with the number of differentiating characters.

Gärtner found by repeated experiments that the respective period of transformation varies in many species, so that frequently a species A can be transformed into a species B a generation sooner than can species B into species A . He deduces therefrom that Kölreuter's opinion can hardly be maintained that "the two natures in hybrids are perfectly in equilibrium." It appears, however, that Kölreuter does not merit this criticism, but that Gärtner rather has overlooked a material point, to which he himself elsewhere draws attention, viz. that "it depends which individual is chosen for further transformation." Experiments which in this connection were carried out with two species of *Pisum* demonstrated that as regards the choice of the fittest individuals for the purpose of further fertilisation it may make a great difference which of two species is transformed into the other. The two experimental plants differed in five characters, while at the same time those of species A were all dominant and those of species B all recessive. For mutual transformation A was fertilised with pollen of B , and B with pollen of A , and this was repeated with both hybrids the following year. With the first experiment $\frac{B}{A}$ there were eighty-seven plants available in the third year of experiment for selection of the individuals for further crossing, and these were of the possible thirty-two forms; with the second experiment $\frac{A}{B}$ seventy-three plants resulted, which *agreed throughout perfectly in habit with the pollen parent*; in their internal composition, however, they must have been just as varied as the forms in the other experiment. A definite selection was consequently only possible with the first experiment; with the second the selection had to be made at random, merely. Of the latter only a portion of the flowers were crossed with the A pollen, the others were left to fertilise

themselves. Among each five plants which were selected in both experiments for fertilisation there agreed, as the following year's culture showed, with the pollen parent:

1st Experiment	2nd Experiment	
2 plants	—	in all characters
3 "	—	" 4 "
—	2 plants	" 3 "
—	2 "	" 2 "
—	1 plant	" 1 character

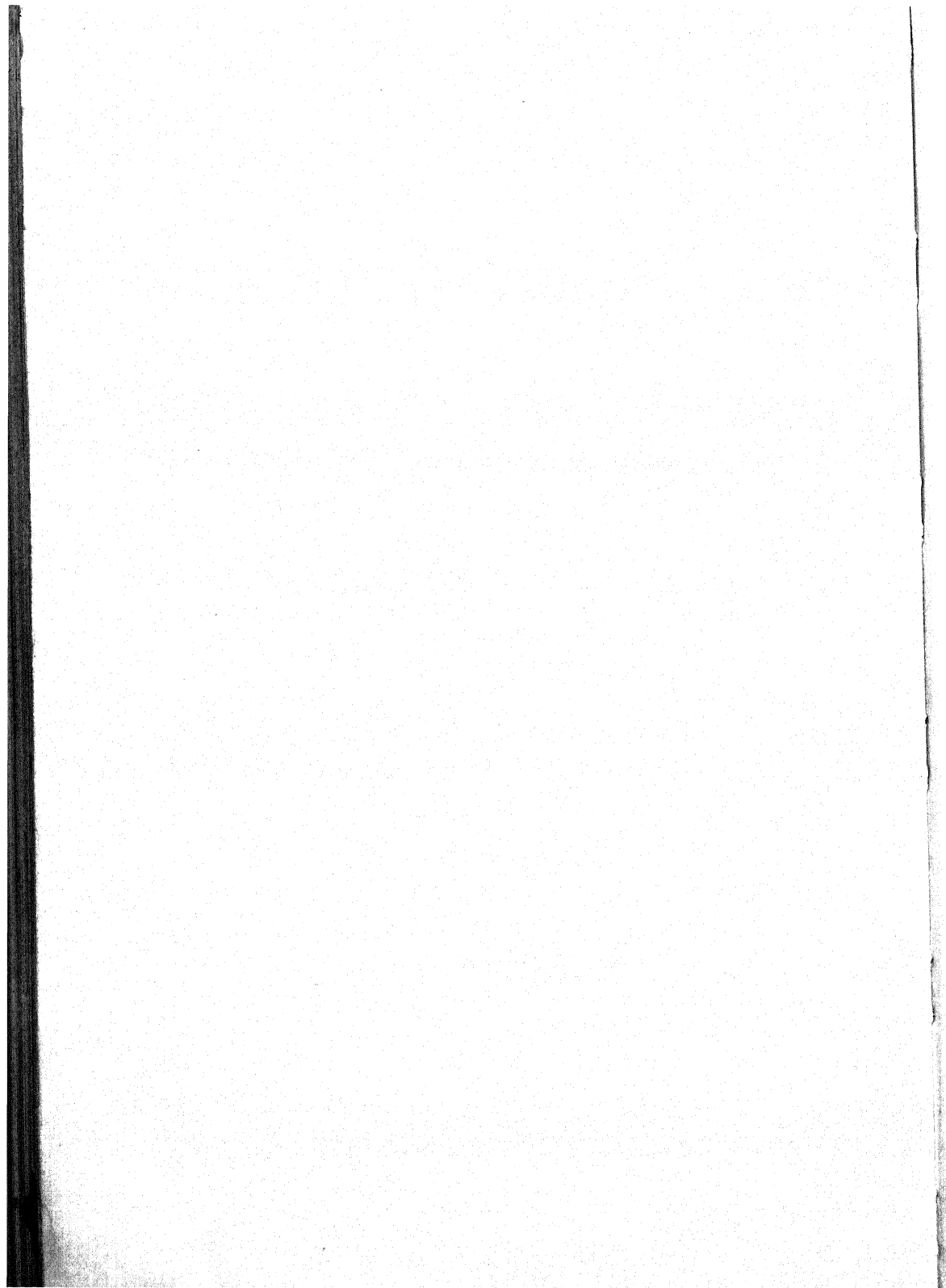
In the first experiment, therefore, the transformation was completed; in the second, which was not continued further, two or more fertilisations would probably have been required.

Although the case may not frequently occur in which the dominant characters belong exclusively to one or the other of the original parent plants, it will always make a difference which of the two possesses the majority of dominants. If the pollen parent has the majority, then the selection of forms for further crossing will afford a less degree of certainty than in the reverse case, which must imply a delay in the period of transformation, provided that the experiment is only considered as completed when a form is arrived at which not only exactly resembles the pollen plant in form, but also remains as constant in its progeny.

Gärtner, by the results of these transformation experiments, was led to oppose the opinion of those naturalists who dispute the stability of plant species and believe in a continuous evolution of vegetation. He perceives¹ in the complete transformation of one species into another an indubitable proof that species are fixed within limits beyond which they cannot change. Although this opinion cannot be unconditionally accepted, we find on the other hand in Gärtner's experiments a noteworthy confirmation of that supposition regarding variability of cultivated plants which has already been expressed.

Among the experimental species there were cultivated plants, such as *Aquilegia atropurpurea* and *canadensis*, *Dianthus caryophyllus*, *chinensis*, and *japonicus*, *Nicotiana rustica* and *paniculata*, and hybrids between these species lost none of their stability after four or five generations.

¹ ["Es sieht" in the original is clearly a misprint for "Er sieht."]



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